Differential frequency-dependent reflex integration of myelinated and nonmyelinated rat aortic baroreceptors

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Fan, Wei, and Michael C. Andresen. Differential frequency-dependent reflex integration of myelinated and nonmyelinated rat aortic baroreceptors. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H632–H640, 1998.—Electrical activation of myelinated (A type) and nonmyelinated (C type) baroreceptor axons (BR) in aortic depressor nerve (ADN) evoked baroreflex changes in mean arterial pressure (MAP) in chloralose-urethan-anesthetized rats. Low stimulation intensities (<3 V) activated only A-type BR electroneurograms (ENG). A-type selective stimulus trains required minimum frequencies >10 Hz to evoke reflex MAP decreases, and the largest MAP responses occurred at 50 Hz and higher. In contrast, high stimulation intensities (18–20 V) maximally activated two volleys in ADN ENG corresponding to A- and C-type BR volleys. High-intensity trains decreased MAP at low frequency (<1 Hz) and largest reflex responses at ≥5 Hz. Capsaicin (Cap) applied periaxonally to ADN selectively blocked C-type ENG volleys but not A-type volleys. Reflex curves with supramaximal intensity during Cap were indistinguishable from the pre-Cap, low-intensity baroreflexes. In comparison, vagus ENG showed graded Cap block of the C-fiber volley (ED50 = 200 nM) without significant attenuation of the A-type volley below 1 µM. However, 100 µM Cap blocked conduction in all myelinated vagal axons as well as C-type axons. Thus Cap is selective for sensory C-type axons only at low micromolar concentrations. Myelinated and nonmyelinated arterial BR evoke characteristically different frequency-response reflex relations that suggest distinct differences in sensory information processing mechanisms.

sensory afferents; blood pressure; myelinated axons; nonmyelinated axons

THE CLASSIC SEPARATION of primary sensory neurons into those with myelinated (A type) and those with nonmyelinated (C type) axons more recently has been linked to differential expression of ion channels, receptors, and neurotransmitters (29). Although such patterning should importantly impact overall reflex function, the distinctions between the reflex contributions of these two neuron classes in any sensory nerve are often blurred by concomitant differences in sensory modality and their central nervous system pathways. In the case of arterial baroreceptors (BR) the basic mechanoreceptive sensory modality is consistent across A- and C-type BR (28), yet a similar dichotomy of cellular properties holds for BR as for somatic sensory neurons. A- and C-type BR have markedly different sensory discharge characteristics (7, 10, 11, 28, 44, 46, 51). On average, A-type BR have lower pressure thresholds and appear to encode pressure with much greater fidelity than C-type BR. Conversely, C-type BR have higher average thresholds, and the relatively sparse and variable discharge of individual BR is often poorly representa-

tive of input pressures (see, e.g., Ref. 55). Although these differences in arterial BR are well established, less is known about the central processing and reflex function from these distinct classes of BR neuron and how these A- and C-type differences impact the constant processing and integration of BR information for dynamic cardiovascular regulation.

The rat is a particularly important animal model for BR and baroreflex work because of the aortic depressor nerve (ADN) (2, 6, 27, 38, 39). This discrete 100-µm-diameter nerve trunk is thought to contain only BR axons, and thus responses to electrical ADN activation in the rat evoke powerful reflex responses that are "purely" BR in origin (19, 36, 37). The rat ADN, then, represents a unique opportunity to study the central processing of BR sensory neurons by bypassing the pressure-encoding step and directly activating the sensory axons using electrical stimuli (21, 22). In other BR-containing nerves such as the carotid sinus nerves and/or the ADN in most other species, the presence of chemoreceptor axons makes responses to electrical nerve activation difficult to interpret unambiguously (see, e.g., Refs. 19, 31, 34, 37). Indeed, such studies in other species have yielded varied baroreflex results (see, e.g., Refs. 5, 17, 18, 34, 35, 42, 47), and no consistent consensus view has emerged of the nature of the integration of A- and C-type BR inputs in baroreflexes.

Our goal in these studies was to systematically characterize the recruitment of A- and C-type BR during graded electrical activation of the rat ADN. We measured the evoked ADN electroneurograms (ENG) to assess afferent axon responses and reflex changes in mean arterial blood pressure to assess central nervous system processing. A-type BR required 10-fold higher frequencies of activation to elicit minimal depressor responses than during full (A + C) ADN activation. Periaxonal capsaicin (Cap) application selectively blocked all C-fiber BR conduction without altering A-type BR reflex responses. Together, the results suggest that the processing of A- and of C-type BR information are fundamentally different with dramatically different frequency-dependent reflex effects.

METHODS

All experiments were conducted using adult male Sprague-Dawley rats (250–450 g; B and K, Kent, WA) in accordance with protocols approved by the University Animal Care and Use Committee.

Baroreflex studies in anesthetized rats. For reflex studies, rats were anesthetized with a combination of urethan (800 mg/kg) and chloralose (80 mg/kg), with supplements of chloralose (10 mg/kg) administered to maintain stable blood pres-
ures. After a ventral midline incision in the neck, the trachea was cannulated and rats breathed spontaneously. Femoral artery pressure was measured (PE-50 tubing filled with heparinized saline). Mean arterial pressure (MAP) was derived from arterial pressure pulses using a Gould Pressure Processor Amplifier and continuously recorded on a pen chart recorder (Gould). In addition, MAP was digitized on-line by a custom software program on a PC-based computer system. With the aid of a surgical microscope, a cervical portion of the left ADN was identified as it joined the superior laryngeal nerve and dissected caudally to where it entered the chest.

Compound action potentials. In separate experiments, two types of ENG experiments, one in situ and one in vitro, were conducted to measure subcomponents of the compound action potentials and conduction velocities evoked by electrical stimulation of either the ADN or vagus nerve. For these experiments, rats were anesthetized with pentobarbital sodium (35–45 mg/kg ip) and intubated for artificial ventilation (Harvard). After a midline ventral thoracotomy, nerves from the left side were exposed.

For the ADN, we initially attempted to dissect and excise a full 20–25 mm of nerve for use in in vitro tests but found that our success rate was too low to proceed. As a compromise, ENG was recorded from ADN in situ. Pulse-synchronous BR discharge was recorded for positive identification. At the rostral end the nerve was sectioned as near to the nodose ganglion as possible, and at the caudal end the nerve was cut very near to where it approached the wall of the aortic arch. The remainder of the nerve was left undisturbed. Bipolar electrode pairs (Teflon-coated Pt-Ir wires) were placed on both the rostral and caudal ends of the nerve, and the conduction path distance between them was measured with an ocular micrometer of a stereomicroscope at ×6 magnification. The nerve and electrodes were then covered with a mixture of petroleum jelly and warm mineral oil for electrical isolation and to prevent drying. One pair of electrodes was used for electrical stimulation, and the other pair recorded the evoked compound action potentials. The stimulating electrodes were connected to a computer-controlled programmable stimulator (AMPI Master 8) through a stimulus isolation unit.

In another set of experiments, the vagus nerve was tested as a surrogated nerve for the more fragile ADN. The vagus was sectioned rostrally near the nodose ganglion and caudally at the level of the heart. The excised nerve was desheathed and placed in a recording chamber that had two pairs of stainless steel wire electrodes fixed at either end of the chamber. These electrode pairs were separated by a fixed distance (20–24 mm). After the vagus was placed on the recording and stimulating electrodes, the nerve and electrodes were covered with a mixture of petroleum jelly and warm mineral oil.

The recording electrodes were connected to a preamplifier (Warner), monitored on an oscilloscope, and digitized at 18 kHz by a microcomputer for averaging and postexperimental analysis. The ENG was amplified (×2,000–5,000) and band-pass filtered (0.1 Hz to 10 kHz). Stimulation protocols were rigorously controlled via the programmed stimulator. Shocks (0.1-ms pulse duration) were delivered every second, and a total of 10 ENG were evoked for computation of averages at each condition. A span of stimulus intensities was tested in all experiments (1, 2, 5, 8, 10, 14, 18, and 20 V). Duplicates using constant current stimulation yielded equivalent results. In the case of the vagus, 24 V was sometimes also tested to assure maximal activation of the measured ENG.

Capsaicin. Cap is reported to selectively inactivate C-fiber axons of sensory neurons without affecting A fibers within the same cutaneous nerves (24). In our ENG experiments, we measured compound action potential volleys before and after application of Cap. Cap was initially dissolved in a concentrated stock solution as a mixture of ethanol (9.5%), Tween (10%), and physiological Krebs solution containing 300 μM Cap that was then diluted with Krebs solution for use. For ADN experiments, a dam of petroleum jelly was constructed along a portion of nerve central to but as close as possible to the stimulating electrodes. This dam created a pool for the placement of a small cotton pledget soaked in Cap solution directly onto the ADN. A similar arrangement was used in the ADN reflex studies. The ADN studies were plagued by the difficulties of obtaining nerve segments of sufficient length for distinguishing the A-fiber volley. We therefore reduced the degree of fine nerve dissection to improve viability of this fragile nerve, and we did not desheath the ADN in these reflex experiments.

Non-specific effects of Cap have been reported (24) but are generally associated with very high concentrations of Cap. We worried about the degree of access of solutions to the ADN without desheathing and thus the degree to which our nominal concentrations in the pledget reflected the actual concentration at the nerve. We turned to the vagus as a surrogated nerve with substantial sensory axon population to enable the Cap concentration-response relation for A- and C-fiber axons over an extended range of concentrations. For the isolated vagus a similar but much larger petroleum jelly dam was created, and solutions of Cap could be placed and readily exchanged on this central portion of isolated, desheathed nerve. In some cases, 2% lidocaine-HCI and/or wash solutions with drug-free Krebs solution were applied. These experiments were run in two overlapping series of concentrations to avoid unduly prolonged protocols. The nerve segment was exposed to control solution (drug-free Krebs solution) followed by solutions containing added Cap at final concentrations of 100, 400, and 1,000 nM in the first series of experiments. Each exposure lasted 20 min. On a different set of vagus nerves, the second series tested control solutions and test solutions containing Cap at final concentrations of 1,000 nM, 10 μM, 30 μM, and 100 μM. Maximum final concentration of vehicle used in Cap delivery (1% ethanol, 1% Tween) had no effect.

Reflex responses. For reflex studies, the cervical portion of the left ADN was isolated for 3–5 mm near its junction with the superior laryngeal nerve. No thoracotomy was performed in these animals. The nerve was placed on stimulating electrodes (bipolar, Teflon-insulated Pt-Ir wires) and covered with the petroleum jelly-mineral oil mixture. On the basis of the ENG results and stimulation intensity tests reported previously (19), two fixed stimulation intensities were used for ADN stimulation in each reflex experiment: low voltage (1.5–2 V) and high voltage (18–20 V). Shocks were 0.1 ms in pulse duration. The high voltage level was sufficient to activate all fiber types in the ADN (see, e.g., Refs. 34 and 36 and Fig. 3), whereas low-voltage stimuli evoked only A-fiber volleys. The low-intensity level was initially verified functionally in each experiment by increasing intensity until a level was reached at which preliminary test trains at 100 Hz elicited marked depressor responses but trains at 1–2 Hz evoked no measurable changes in blood pressure. Stimulus trains lasting 60 s were tested for selected fixed frequencies: 1, 2, 5, 10, 20, 50, 100, and 200 Hz. The order of application of frequencies was random. Stimulus trains were given 3–5 min apart, which was sufficient time to allow full recovery. MAP signals from the pressure processor were displayed on the pen recorder and digitized at 20 Hz for off-line analysis. In many cases heart rate was also derived from the pulsatile pressure signal, but because preliminary analysis showed that heart
rate and MAP responses were qualitatively very similar, only MAP was used for final analysis and for reporting here. In the Cap reflex studies, we guarded against potential nonspecific actions of Cap on A-type responses by periodically testing the high-frequency, low-intensity reflex responses. We tested repeatedly using low-intensity, high-frequency volleys. In any experiments in which this response decreased, the results were discarded.

Analysis. For the ENG experiments, conduction velocities were calculated by dividing the measured nerve length by the time elapsed from the stimulus artifact to the initial edge of a given subcomponent. Mean ENG were collected by a custom data acquisition program from 10 successive samples of individual traces. These mean ENG were then ported to a graphic analysis program (Origin, Microcal Software) for plotting, and magnitudes of the subcomponents of the mean ENG (A wave and C wave) were assessed by full wave integration of the respective segments. Plots (intensity vs. time) were made of individual mean ENG at each intensity or condition tested, and mean integrated areas for the A- and C-wave subcomponents of the ENG were plotted against intensity for comparison. Average data are displayed as means ± SE.

For reflex experiments, MAP responses were calculated as changes in MAP relative to the prestimulation value for each test (ΔMAP). The control value was the average of MAP values sampled for 10 s preceding the stimulus. This control MAP was subtracted from the mean reflex response values averaged between 50 and 60 s of the sustained stimulation period. Across experiments, average reflex response relations were plotted as the mean MAP against stimulation frequency for low- and high-voltage stimuli and in the presence of Cap. Comparisons were made using analysis of variance, and in some cases post hoc pairwise comparisons were done using Scheffé’s test. P values <0.05 were considered significant.

RESULTS

Cross sections of the ADN of the rat show BR axons with and without myelination in an A-to-C ratio as high as 1:9 (2, 20, 26). To test the range of conduction velocities and intensity-dependent recruitment, we recorded ENG responses evoked by graded single shocks. At the lowest stimulation intensities, a very short latency volley appeared in the ADN ENG. The early volley corresponded to a conduction velocity of ~10 m/s, which is typical of lightly myelinated A fibers (Fig. 1). As stimulation intensity was increased, the amplitude of the A-fiber volley increased over a very narrow range so that, above 2 V, this early volley generally did not change significantly in amplitude (n = 6 nerves, P > 0.100) or form (Fig. 2). At stimulus intensities >5 V, a long-latency subcomponent appeared in the ENG that corresponded to conduction velocities slower than 0.5 m/s (Figs. 1 and 2) and within the C-fiber range. An intermediate volley corresponding to a conduction velocity of ~5 m/s was discernible in some ADN and is consistent with a class of very lightly myelinated or B-fiber sensory axons (16, 53). Further increases in stimulus intensity up to ~15 V generally increased the amplitude of the late C-fiber wave (n = 6 nerves, P < 0.05; Figs. 1 and 2), indicating recruitment of increased numbers of C fibers. Beyond this, the ENG was constant without further changes in the A- and C-fiber waves, indicating activation of all axons in the nerve.

Thus, on average, threshold intensity for ADN A fibers was ~1 V and threshold intensity for C fibers was >5 V (Fig. 2). Thus, low-intensity stimuli allow us to examine reflex responses to selective activation of myelinated BR without activating C-type BR. These low intensities, however, may not activate all A-type fibers in the ADN. High, supramaximal intensities dearly activate both A- and C-type axons together (Figs. 1 and 2).

Using two levels of stimulus intensity, a low A-type selective level and a high A + C maximal level, we measured baroreflex MAP responses evoked by trains of ADN activation. Initial reflex testing suggested that low-intensity stimuli (~3 V) elicited distinctly different

![Fig. 1. Stimulus intensity-dependent recruitment of myelinated and nonmyelinated compound action potentials in aortic depressor nerve (ADN) electroneurogram (ENG). ADN was left in situ, and peripheral and central ends were cut and freed for stimulation and recording. Short-latency volleys were present at all intensities tested. At ~8 V, a late component corresponding in conduction velocity (CV) to C fibers appeared (dashed vertical line) and increased in amplitude with increasing stimulus intensity. Stimulus shock duration was 100 µs, and single shocks were delivered each second.](http://ajpheart.physiology.org/)
frequency baroreflex response relations than supra-
maximal intensities (18–20 V). Baroreflex frequency
response relations to ADN stimulation were con-
structed at these two intensity levels over an extended
range of fixed frequencies. At low intensities, ADN
stimulus frequencies of up to 10 Hz evoked no signifi-
cant changes in MAP (Fig. 3, A and D). Above 10 Hz at
the same low intensity, ADN stimulus trains evoked
frequency-dependent decreases in MAP. Such low-
intensity, high-frequency reflex responses in blood pres-
sure reached upper plateau values, on average (Fig. 4),
at frequencies >50 Hz (i.e., 50, 100, and 200 Hz
responses were similar, n = 9 nerves, P > 0.865).
Increasing stimulus intensity to high levels (18–20 V)
in these same nerves evoked substantial baroreflex
decreases in MAP now at frequencies as low as 1 Hz
(Fig. 3, B and D). At these high intensities, the reflex
response increased with increases in stimulation fre-
quency up to only 5 Hz. Beyond 5 Hz at high stimulus
intensities, further increases in frequency did not fur-
ther increase the reflex MAP response (n = 9 nerves,
P > 0.284) and the average frequency-response relation
reached a plateau response that was maintained
through 200 Hz (Fig. 4). Thus, on average, half-
maximal reflex responses occurred at ~2 Hz with
maximal stimulus intensities, whereas half-maximal
responses required ~20 Hz at low intensities. Thus
these results demonstrate that activation of A-type BR
alone has a distinctly different frequency response
relation than when coactivated with C-type BR.

The vanillanoid Cap activates a cation-selective ion
channel found only in C-type sensory neurons and in its
continued presence leads to an inactivation of these
neurons (24, 54). Because the Cap sensitivity of arterial
BR is unclear, we designed experiments to test the
susceptibility of arterial BR to Cap as a way of testing
A-selective responses at maximal activation intensi-
ties. We directly measured the axonal conduction of
myelinated and nonmyelinated BR in the ADN by
recording ENG before and after Cap. Periaxonal appli-
cation of Cap depressed and then completely blocked
the slow-conducting compound action potential volley
(Fig. 5). The fast-conducting A-fiber component was
preserved when the C-fiber volley had disappeared
(Fig. 5). The lowest effective Cap concentrations (1 µM)
that completely blocked the C volley in ADN required
60–70 min to achieve full blockade (n = 3 nerves). In
these experiments, there was no significant change in
the integral of the ADN A-fiber volley during applica-
tion of 1 µM Cap (n = 3, P = 0.21). However, if higher
concentrations (50 µM) were used, similar differential
blockades of C-type volleys were observed but they
were established more quickly (~10 min, Fig. 5). Given
this observed selective blockade of the ADN ENG, we
used Cap to test the baroreflex responses to maximal

Fig. 3. Reflex changes in mean arterial pressure (MAP)
for a single experiment in response to low- and high-
intensity stimulation of ADN at different frequencies.
Detectable MAP responses to low-intensity ADN stimu-
lation (A) occurred only at 20, 50, and 100 Hz, whereas 2
and 10 Hz failed to alter MAP. At high intensities (18 V;
B), 2 Hz evoked a large reflex decrease in MAP. Expos-
ure of ADN to capsaicin (Cap; 30 µM; C) placed just
central to stimulation site greatly reduced 2- and 10-Hz
MAP responses to high-intensity stimulation, whereas
high-frequency responses were preserved. Double arrow-
headed horizontal lines (A–C) indicate 60-s stimulation
interval. D: frequency-response relation for this single
experiment for low intensity, high intensity, and high
intensity with Cap application; plot includes additional
data not displayed in A–C. Stimulation was unilateral
on central end of sectioned ADN. Stimulus shock dura-
tion was 100 µs. ΔMAP, change in MAP from prestimu-
lus MAP.

Fig. 4. Average reflex ΔMAP for 9 rats in response to different
frequencies of ADN stimulation. Frequency-response relations are
for low intensity, high intensity, and high intensity with Cap applica-
tion. Note that during Cap, high-intensity responses overlie control,
low-intensity response values. Values are means ± SE.

Fig. 5. Reflex changes in mean arterial pressure (MAP) for a single
experiment in response to low- and high-intensity stimulation of ADN at
different frequencies. Detectable MAP responses to low-intensity ADN
stimulation (A) occurred only at 20, 50, and 100 Hz, whereas 2
and 10 Hz failed to alter MAP. At high intensities (18 V;
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experiment for low intensity, high intensity, and high
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on central end of sectioned ADN. Stimulus shock dura-
tion was 100 µs. ΔMAP, change in MAP from prestimu-
lus MAP.
activation of the A-type BR population of ADN in the absence of a conducted C-type BR component. We applied Cap in an identical manner to produce axonal blockade and stimulated ADN at the various test frequencies. Periaxonal application of Cap to a portion of the nerve central to the stimulation site on the ADN greatly reduced the reflex MAP responses to low frequencies (<10 Hz) despite supramaximal intensity nerve stimulation (Fig. 3, compare results at identical frequencies in B with C). On average (Fig. 4), the low-frequency responses to high-intensity stimulation were completely suppressed and the baroreflex frequency-response relation during Cap overlapped the low-intensity control curve (n = 4 nerves, P = 0.617). Such results suggest that 1) the low-intensity (<3 V) stimuli of our reflex series functioned as a near-maximal stimulus and 2) Cap did not significantly impair A-type BR conduction under these conditions, a finding consistent with the ENG results. Clearly, the use of Cap or low stimulus intensity can effectively isolate the portion of the ADN baroreflex response derived from A-type BR. Selective stimulation of A-type BR evokes somewhat smaller total MAP responses than high-voltage (A + C) stimulation (n = 9 nerves, P < 0.0001; Fig. 4). Thus simultaneous activation of C-type BR with A-type BR augments the total reflex response.

The concentrations of Cap required to block the ADN quickly were relatively high—in the range of 10–30 µM—and we were concerned about the specificity of action of such concentrations. The small total nerve trunk diameter and relative fragile nature of the ADN, in our experience, made clear access to the nerve difficult. Attempts at full-length dissections and regional desheathing of the ADN were not reproducibly successful. These attempts most often resulted in functional nerves of insufficient length to resolve the A-fiber volley (requiring >20 mm). Resolution of both waves is critical to test for the A-type/C-type differential effects of intensity and Cap. We attribute these dissection failures primarily to the difficulty in surgical isolation of the ADN. To circumvent this difficulty, some connective tissue was generally left in place surrounding the ADN during these dissections to minimize surgical damage of the nerve. This may have impeded diffusion of Cap to the axons.

For comparison to ADN experiments with Cap, we conducted similar ENG studies on excised cervical-thoracic segments of the vagus nerve. Our motivation was to determine more precisely the concentration-response relation for cranial sensory axons and the relation between Cap concentrations that selectively blocked axonal conduction in C-type fibers and so-called nonspecific effects of Cap at high concentrations that depressed A-type conduction. Desheathing of the vagus nerve was routinely successful in this much larger nerve. Long segments of vagus nerve (>20 mm) were dissected, excised, desheathed, and mounted in a stimulation recording chamber. The larger size and in vitro isolation of the vagus assured more unrestricted access of applied solutions to the axons and permitted a more rigorous control of Cap exposure. Stimulation of the vagus evoked short- and long-latency volleys that were qualitatively similar to ADN in their stimulus intensity dependence of the A- and C-fiber volleys (Fig. 6). A-fiber volleys (7–20 m/s) were present at minimal stimulus intensities, and a C-fiber volley (<0.8 m/s) was recruited at higher intensities. On average (n = 12 nerves), the A-fiber volleys were nearly fully activated at 2 V and the C-fiber volley had threshold responses at 8 V (Fig. 7). These vagus results were generally similar to the intensity dependence of subtype volleys in the ADN (Fig. 2).

Application of as little as 100 nM Cap to a section of the excised vagus nerve selectively and reversibly depressed the C-fiber volley (Fig. 8). This depression was steeply concentration dependent at the lower range but markedly less so between 1 and 100 µM (Fig. 9). There was little discernible effect on the short-latency A-fiber response (Fig. 8). On average (n = 7 nerves),
50% block of the C-fiber volley was produced at 200 nM Cap (Fig. 9). The A-fiber volley was not significantly changed \((n = 12, P > 0.05)\) by increasing Cap up to 1 µM. However, when the concentration was increased 10-fold or greater to 10 µM Cap, frank depression of the A-fiber volley appeared. As with the ADN, onset of blockade of the vagal C-fiber volley was noticeably more rapid with higher Cap concentrations. The effects of Cap \((<10 \mu M)\) were reversible by washing the exposed segment in drug-free Krebs solution for 20 min, and lidocaine rapidly blocked the ENG completely (Fig. 8). Thus these results are consistent with two types of effects of Cap that are separable by concentration, a C-selective blockade at low concentrations \((100-1000 \text{ nM})\) and a nonselective or nonspecific blockade at very high Cap concentrations that blocks all conduction in the vagus nerve. Overall, the lower-range Cap results \((0.1-1 \mu M)\) in the vagus nerve were quite comparable to the more limited series of ADN experiments in which prolonged exposure times were required to be effective at relatively low concentrations (Fig. 9).

**DISCUSSION**

Most arterial BR \((-90\%)\) have C-fiber axons \((9, 16)\) including those innervating the aortic arch in the rat (2). These C-type BR in the rat behave very differently from myelinated, A-type BR \((7, 48, 49, 51)\), and similar...
differences are found in other species (9–11, 25, 43, 45, 50). Variability of discharge frequency (interspike interval) and in pressure transduction properties (threshold, sensitivity, and reproducibility) is a hallmark of C-type BR (see, e.g., Refs. 10 and 11). Interestingly, this functional variability of BR seems to be correlated with differences in the cellular characteristics of C-type sensory neurons generally such as the expression of ion channels, neurotransmitter receptor types, and neurotransmitters compared with A-type sensory neurons (23, 29, 40, 41). To understand the potential central nervous system impact of these subtype differences, it is critically important to define fully the reflex responses to their activation.

Functionally, C-type BR have been described as antihypertensive and as important primarily in evoking reflex responses at elevated blood pressures (see, e.g., Refs. 1, 34, 49). From single-fiber studies of BR (25, 49), however, it is clear that a substantial portion of the C-type BR population has a threshold at or below normal resting pressures and conversely that many A-type BR have thresholds well above resting pressures and are unlikely to be active at rest (2, 3). Thus a mixture of A- and C-type BR are simultaneously active at rest, and newly active neurons of both subtypes are recruited as pressure rises. This makes it critically important to understand the nature and mechanisms of the individual actions of these subtypes as well as the nature of their interactions in the central nervous system.

In the present studies, we directly demonstrated the intensity-dependent recruitment of A- and C-type BR axons by recording ADN volleys and calculating the corresponding conduction velocities. At the lowest intensities, only a fast-conducting (>0.5 m/s) volley was detected. A second broad and slow-conducting wave appeared at greater intensities that corresponded to C-fiber conduction velocity (>0.5 m/s). Conducted volleys of A-type BR axons were 1 nearly maximally activated by relatively low-intensity stimuli that were insufficient to evoke C-type volleys in the ENG and 2) not significantly affected by a low-concentration peripheral Cap treatment that fully blocked the C-fiber volley in the ADN. Thus A-type and C-type BR of the rat ADN show differential stimulus intensity recruitment and pharmacological sensitivity to Cap that are similar to those of many other sensory neurons (24).

The baroreflex responses attributed to activation of these two subtypes of BR appear to have distinctly different frequency-response characteristics. Activation of A-type volleys in the ADN evoked reflex changes in MAP only at relatively high frequencies (>10 Hz) compared with responses evoked by stimuli that recruited C-type BR volleys (A + C, 1 Hz). The fact that no responses resulted from low frequencies (<10 Hz) of A-selective stimuli (either at low stimulus intensities or at maximal intensities but with C-type conduction blocked by Cap) suggests minimal functional overlap in the submaximal regions of A- and C-type frequency-response relations for MAP. A + C-type responses reached a maximum reflex MAP response at frequencies (~5 Hz) below the minimum frequency required to elicit reflex MAP responses from A-type BR alone. Thus, when all BR in the ADN are activated, the reflex MAP response is maximal and sustained over a wide range of frequencies from ~10 to 200 Hz. Interestingly, the ranges for frequency-graded reflex responses (1–5 Hz for A + C type and 10–100 Hz for A type) correspond quite closely to the graded range of discharge rates commonly recorded from single-fiber preparations of the two BR subtypes, respectively, in response to pressure stimulation (see, e.g., Ref. 28). The A-type BR alone are capable of separately producing some 70–80% of the combined maximal MAP response. Thus summation of A- and C-type information is clearly nonlinear or possibly occlusive at maximal activation frequencies for MAP responses. Generally similar A + C-type BR frequency-response relations at maximum stimulus intensity have been reported for blood pressure responses in the decerebrate rat (36) and for A- and C-type components in inhibition of renal sympathetic activity in the rat (33) and rabbit (32). Such stimuli activate all axons and may represent supramaximal inputs that may influence their central summation properties across BR subtype.

As we have noted previously (19), the character of electrical stimulation and the action potential volleys that are produced are inherently different from those of natural pressure stimulation of BR. Electrical shocks recruit fibers based on axon characteristics that are unlikely to be strictly related to pressure sensitivity and pressure threshold. In addition, the volleys of action potentials evoked by electrical stimulation have a degree of synchrony that does not occur with natural stimulation. Thus, although our previous results have shown that within the critical frequency ranges that discriminate between A- and C-type BR axons (1–20 Hz) both fiber types conduct faithfully (19), it must
always be recognized that these sensory inputs are inherently different from those arising from pressure stimuli. Finally, we have not graded within subtypes either the A-type or C-type responses, so suramaximal stimuli could obscure true compromise in function of some baroreceptor axons. Despite these qualifications, electrical stimulation is an essential tool to understanding cellular characteristics at the level of single neurons and is the basis for most electrophysiological strategies for studying these neurons. Thus understanding these stimuli is essential to probing the cellular mechanisms that underlie these fundamental differences between A- and C-type BR neurons and the neural circuits to which they contribute.

Cap has a long and varied history as a tool to study sensory neurons, and there has been much discussion about selectivity and specificity of its actions (24). Cap can activate and then block conduction in a subset of C-fiber vagal afferents including those functionally defined as cardiovascular (10) and respiratory in origin (12), but arterial BR had never been tested previously. In our isolated preparations, C-fiber volleys of both ADN and vagus were reversibly blocked by periaxonal Cap application, whereas A-fiber volleys were unaffected up to moderate concentrations (<1 µM). Thus we found equivalent functional isolation of the A-type baroreflex with either low concentrations of Cap to block C-type conduction or low-intensity stimulation to recruit only A-fiber volleys (see Fig. 2). However, the vagal segments tested contained both visceral sensory axons (cardiac, cardiopulmonary, gastrointestinal, etc.) and motor fibers, and these efferent fibers include C-type axons (see, e.g., Ref. 30). Collectively, our ENG results are consistent with a relatively uniform Cap sensitivity among C-type sensory axons including arterial BR (e.g., ADN) as well as a mixture of modalities of other visceral sensory afferents (vagus). Although we did not find a Cap-insensitive subpopulation of C-type fibers in ADN as reported in other nerves (8), a relatively small (<20%) component of the vagus ENG was clearly Cap resistant (conduction block only at Cap > 10 µM). We speculate that these Cap-resistant axons are effenter C-type fibers (30). Blockade of this resistant C-type component, however, coincided with the high Cap concentration range, which reduced the amplitude of the A-fiber volley of the vagus, and thus these high concentrations may be better termed “nonselective.” This resistant portion may represent the population of neurons that lack high-affinity Cap-sensitive receptors (4, 24) but are blocked by nonspecific Cap actions as indicated in binding studies.

The present studies suggest that myelinated and nonmyelinated arterial BR have distinctly different frequency-response relations. Although baroreflex responses to low frequencies of electrical ADN activation (<10 Hz) are entirely dependent on the presence of C-type BR conduction, even low rates of activation of C-type BR evoke powerful depressor responses. Conversely, myelinated BR require much higher frequencies for equivalent reflex responses. At an operational level, our results indicate the need for caution when applying electrical stimulation in baroreflex studies because, depending on the frequency of stimulation even at a fixed intensity, reflex responses could be caused by a varying mix of A- and C-type BR afferents and this in turn could complicate interpretation of the results. For example, a fairly common experimental protocol uses a fixed intensity of stimulation with variable frequency to construct a baroreflex relation. Such a derived relation will be variably mediated by a changing pool of A- and C-type BR. Interestingly, the distinctive, subtype-selective rate dependence for baroreflex responses matches correspondingly the predominant ranges of discharge frequencies commonly observed in the respective BR subtypes (~1–5 Hz for C type and 10–100 Hz for A type; see, e.g., Ref. 28). More work will be required to resolve the mechanisms responsible for these differences in BR-subtype reflex efficacy. Emerging results from our lab using anodal blocking techniques to separate A- and C-type inputs from the ADN confirm that there are fundamental, frequency-dependent differences in BR-subtype processing across baroreflex pathways. Many possibilities exist, ranging from anatomic differences in convergence (13–15) to possible differences in expression of ion channels or neurotransmitters. In analogous areas of spinal cord, work on processing of somatosensory information suggests that C-type sensory neurons release a diversity of neuropeptides that A-type sensory neurons do not (52), and such differences could be critical in central autonomic pathways.

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