Aortic depressor nerve unmyelinated fibers in spontaneously hypertensive rats

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Fazan, Valéria Paula Sassoli, Helio Cesar Salgado, and Amilton Antunes Barreira. Aortic depressor nerve unmyelinated fibers in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 280: H1560–H1564, 2001.—The objective of the present study was to compare the morphology of the unmyelinated fibers in the aortic depressor nerves (ADN) of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). In anesthetized rats, the ADN was identified by its spontaneous activity synchronous with the arterial pulses. Thin sections of the proximal and distal segments of the ADN were analyzed by electron microscopy, and a morphometric study of the unmyelinated fibers and Schwann cells was performed. The proximal segments of WKY and SHR ADN contain an average of 335 ± 68 and 130 ± 14 unmyelinated fibers, respectively (P < 0.05), and the distal segments contain an average of 337 ± 46 and 242 ± 77 unmyelinated fibers, respectively (P < 0.05). The distribution of the diameters of unmyelinated fibers was unimodal for both strains, with the histogram from the SHR significantly shifted to the left. Because the unmyelinated fibers play a role in the tonic inhibition of the medullary vasomotor centers, especially in the presence of hypertension, the morphological differences observed in the ADN from SHR may account, at least in part, for the blunt baroreflex of SHR.

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METHODS

Experiments were performed on adult male and female SHR (n = 9) with well-established hypertension (>24 wk old) and age-matched WKY (n = 13) weighing 250–400 g that were anesthetized with pentobarbital sodium (Nembital, 40 mg/kg ip). The left ADN, when presented as a separate strand (in ~30% of the animals for both strains), was carefully isolated from the connective tissue and placed on a bipolar stainless steel electrode, and electrical activity was recorded to ascertain that the nerves studied morphologically were baroreceptor nerves (5). The electrophysiological parameters of the nerves studied here have been previously described and discussed (5).

After the electroneurographic recording, the proximal segment (close to the nodose ganglion) of the ADN was cut and marked with a thread, ~20 mm of the nerve were removed, and proximal and distal (close to the aorta) segments of the nerves were prepared for transmission electron microscopic study as described previously (5, 6). The unmyelinated fibers were studied under a transmission electron microscope (Hitachi-7000). Thin sections (30–50 nm) were mounted on
200-mesh copper grids and stained with lead citrate and uranyl acetate. One micrograph of the whole nerve and at least three micrographs for SHR nerves and five micrographs for WKY nerves at 5,000 times the original magnification were obtained randomly for each nerve without overlap of microscopic fields. The negatives obtained were scanned with the use of the Imapro QC30–3200 flated scanner, and the images were stored on a Power Macintosh for later analysis. The area and diameter of each unmyelinated fiber inside the fascicles were measured with image analysis software (KS400, Kontron 2.0; Eching Bei München, Germany). The density of the unmyelinated fibers was calculated, and the total number of unmyelinated fibers was inferred on the basis of the density results. The total number of fibers (myelinated and unmyelinated) was obtained, and total fiber density was also calculated. The ratio of unmyelinated to myelinated fibers was obtained. The percentage of the total fascicular area occupied by the unmyelinated fibers and by both types of fibers (myelinated and unmyelinated) was also calculated. The number of Schwann cell nuclei present in each transverse section of the depressor nerve was counted and their density calculated. The number of unmyelinated axons associated with each Schwann cell or portion thereof (hereafter called a Schwann cell unit) was determined in seven WKY and five SHR nerves as previously described (6).

Histograms of the size distribution of the unmyelinated fibers were constructed and separated into class intervals increasing by 0.1 μm.

Morphometric parameters were compared by nonparametric tests as described previously (5). Differences were considered significant if P < 0.05.

RESULTS

Number and size of fibers. WKY depressor nerves were found to contain, on average, 335 ± 68 (80% of the total) unmyelinated fibers proximally and 337 ± 46 (82% of the total) distally. The SHR nerves contained, on average, 130 ± 14 (64% of the total) unmyelinated fibers proximally and 242 ± 77 (76% of the total) distally. The total number of fibers was 418 ± 65 proximally and 410 ± 43 distally for WKY nerves. For SHR nerves, the total number of fibers was 203 ± 14 proximally and 316 ± 83 distally. There was no significant difference between segments for either group of animals. When the data for SHR and WKY were compared, WKY nerves were found to have a larger total number of fibers (P = 0.001) and a larger number of unmyelinated fibers (P = 0.007). The total density of fibers in WKY nerves was 244 ± 41 fibers/mm² of nerve, and the unmyelinated fiber density was 200 ± 43 fibers/mm² of nerve. For SHR nerves, the total density was 175 ± 15 fibers/mm² of nerve, and the unmyelinated fiber density was 118 ± 18 fibers/mm² of nerve. Statistical analysis showed no difference in total fiber density or unmyelinated fiber density when segments (proximal vs. distal) and groups (WKY vs. SHR) were compared. The ratios of unmyelinated to myelinated fibers were 6 ± 2 for WKY nerves and 3 ± 1 for SHR nerves, with no statistically significant differences between segments or groups.

The overall population of unmyelinated fibers for both strains had a unimodal size distribution (Fig. 1). The mean diameter of unmyelinated fibers was 0.7 ± 0.01 μm for both nerve segments in WKY rats and 0.6 ± 0.01 μm for both segments in SHR, and this difference was statistically significant. Unmyelinated axons as small as 0.2 μm and as large as 1.5 μm were present in all SHR nerves, whereas for WKY nerves, the smallest axon was 0.3 μm and the largest one was 2.0 μm in diameter. About 72% of the unmyelinated fibers of WKY nerves and 86% of the unmyelinated fibers of SHR nerves were smaller than 0.8 μm, and 19% of the WKY and only 8% of the SHR nerve unmyelinated fibers were larger than 1.0 μm in diameter. The size distribution of the unmyelinated axons showed a considerable overlap with the corresponding distribution of myelinated axons (5), and the largest WKY unmyelinated axons (2.0 μm) were larger than 77% of the myelinated axons. For SHR nerves, the largest unmyelinated axons (1.6 μm) were larger than 70% of the myelinated axons.

The mean percentage of the fascicular area occupied by unmyelinated fibers was 12 ± 3% for WKY nerves and 5 ± 1% for SHR nerves. The difference between segments was not significant, but the difference between strains was evident. The mean percentage of fascicular area occupied by fibers (myelinated and unmyelinated) was 54 ± 5% in WKY rats and 42 ± 3% in SHR, also reaching a statistical difference.

Schwann cells. An average of 46 ± 3 Schwann cell units were found in each WKY and SHR nerve cross section. Each Schwann cell enveloped 1–10 or more unmyelinated axons, with an average of 3.3 ± 0.6 and 1.8 ± 0.1 axons/unit for WKY and SHR nerves, respectively. No Schwann cell was devoid of axons. An average of 39 ± 6% of the unmyelinated axons were not accompanied by other axons in their Schwann cell envelopment in WKY nerves (Fig. 2). For SHR nerves,
59 ± 4% of the unmyelinated axons were present in single Schwann cell units (Fig. 2). For both strains, all axon sizes were represented in single Schwann cell units, but the larger ones predominated. When the same Schwann cell enveloped two or more axons, the axons were not in contact with each other. Instead, each axon was located in a separate Schwann cell trough. An average of 19 ± 3 and 22 ± 3% unmyelinated axons of WKY rats and SHR, respectively, were enveloped in groups of two, and smaller proportions of axons were enveloped in larger groups. The same Schwann cell often enveloped axons of different sizes. In each proximal or distal segment of the WKY nerve, there were, on average, 11 ± 1 Schwann cell nuclei (6 ± 0.5 cell/mm² of fascicular area). For SHR nerves, proximal segments had an average of 10 ± 2 Schwann cell nuclei (7 ± 1 cell/mm² of nerve), and distal segments had an average of 14 ± 2 Schwann cell nuclei (11 ± 1 cell/mm² of nerve), attaining a significant difference.

DISCUSSION

Information about the number of unmyelinated fibers of the ADN in WKY and SHR was first reported by Andresen et al. (2). These authors studied only the distal segment of the nerve (close to the aorta) and found no significant differences either between number of fibers or in ratio between unmyelinated and myelinated fibers. Their results differ from ours, because we observed a larger number of unmyelinated fibers in the ADN of WKY rats in both proximal and distal segments. These differences might be ascribed to animal age differences and to a different methodological approach to nerve preparation, such as different buffers and fixative solution, different staining methods, and visual inspection for counting fibers. Although there was a wide variability in the data reported by Andresen et al. (2) compared with ours, their results showed a greater variability for SHR when compared with WKY rats, and this result is in agreement with our previous (5) and present studies. Our previous report of the number of myelinated fibers of WKY and SHR did not show differences between the two strains (5), but in the present study, differences were found in the total number of fibers and were attributed to the difference in the number of unmyelinated fibers. The total number of ADN fibers for Wistar rats (438 fibers) as well the percentage of the unmyelinated fibers (81%) described previously (6) are comparable with the numbers obtained here for WKY but differ from those obtained for SHR.

There is no report about the size distribution of the unmyelinated fibers of WKY and SHR. Brown et al. (3) reported that the unmyelinated fibers of the ADN of Wistar-Lewis rats vary from 0.25 to 0.9 μm in diameter. Our previous report showed that the ADN of Wistar rats have unmyelinated fibers with an average diameter of 0.5 ± 0.1 μm, with unimodal distribution (6). In the present study, a unimodal distribution of the unmyelinated fibers for SHR and WKY was found, and the statistical analysis showed that the unmyelinated fibers of the ADN of SHR have smaller diameters than those from WKY. The unimodal distribution for unmyelinated fibers in peripheral nerves is considered a

![Fig. 2. Cross section of an SHR (A) and a WKY rat (B) depressor nerve showing typical Schwann cell units. One Schwann cell can envelop axons of different sizes. Each Schwann cell enveloped 1–10 or more unmyelinated axons, with an average of 3.3 ± 0.6 and 1.8 ± 0.1 axons/Schwann cell unit for WKY and SHR nerves, respectively. Note that in the SHR nerve, most of the Schwann cell units have a single axon (arrows), whereas in the WKY nerve, Schwann cell units are larger.](http://alpharef.physiology.org/.../by10.220.32.246 on April 5, 2017)
normal feature (8, 15), whereas a bimodal distribution has been described in pathological conditions (20). Although there was a difference between strains in the number of unmyelinated fibers, the comparison of the unmyelinated fiber density of the ADN of WKY rats and SHR did not show a significant difference. This is due to the fact that the ADN of SHR are generally smaller compared with WKY (5).

The ratio between unmyelinated and myelinated fibers is considered ideal when it is equal to a minimum of 3:1 (7, 15). The unmyelinated-to-myelinated fiber ratio was 6:1 for WKY, whereas it was closer to the minimum normal value (3:1) for SHR. Taking into account these parameters, our results differ considerably from those obtained by Andresen et al. (2), who found a 10:1 ratio for both strains, but with wider variability (standard errors > 35%). These differences might be due to the small number of animals examined in that study, i.e., five animals. In the present study, the variability observed with 9 SHR and 13 WKY rats was considerably smaller (standard errors < 25%).

Measurement of the conduction velocity of ADN unmyelinated fibers indicated a speed of 0.5–2 m/s (3, 4). In the present study, we did not measure the conduction velocity of the depressor nerves, but, using the scaling factor of Gasser (8) for calculating the conduction velocity of unmyelinated fibers in meters per second (1.7 times the axonal diameter), we calculated that WKY unmyelinated fibers conduct at 0.3–3.4 m/s, whereas those of SHR conduct at 0.3–2.5 m/s. These data suggest that the unmyelinated fibers from SHR might be conducting baroreceptor impulses to the central nervous system more slowly than in the WKY rats.

Thorén et al. (21) investigated the electrophysiological characteristics of the unmyelinated fibers of the ADN of rats and found that the discharges during normotension are sparse, especially from those with irregular activity. The authors concluded that these fibers contribute significantly to the inhibition of the vasomotor medullary centers. Gonzalez et al. (9) and Ohta and Talman (16) showed that the pressor response to electric stimulation of the ADN under anesthesia was reduced in SHR compared with WKY. Hence, the authors suggested that this attenuation could be due to differences in afferent fibers. Our data show a smaller number and a smaller diameter of unmyelinated fibers for the ADN of SHR, providing an anatomical basis to explain, at least in part, the blunted baroreflex of SHR.

Takeda et al. (19) studied the ADN function of SHR by means of ADN deafferentation, providing evidence of a reduced sympathoinhibitory response to the elevation of blood pressure caused by phenylephrine infusion. They suggested that an impaired afference of the baroreflex may contribute to the development of hypertension in the SHR. Another possible mechanism to explain the development of hypertension in SHR is an augmented sympathetic activity in these animals. Because the unmyelinated fibers play a role in the tonic inhibition of the medullary vasomotor centers, especially in the presence of hypertension (21), we may speculate that a reduced number of unmyelinated fibers in SHR depressor nerves could play a role in the sympathetic hyperactivity shown by these animals. Although Judy and Farrel (10) have suggested that the sympathetic hyperactivity of the SHR may be associated with activation of a higher central nervous system excitatory center that is additive to the medullary sympathetic outflow, the results of Gonzalez et al. (9) in decerebrate rat indicate that the influence of hypothalamus and other higher central nervous system structures is not necessary for increased sympathetic activity in the SHR.

In conclusion, the morphological differences observed in the ADN from SHR compared with those from WKY rats may account, at least in part, for the decreased baroreceptor sensitivity observed in this chronic hypertensive model.

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