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VAGAL AFFERENT INNERVATION AND REMODELING IN THE AORTIC ARCH OF YOUNG-ADULT FISCHER 344 RATS FOLLOWING CHRONIC INTERMITTENT HYPOXIA

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Abstract—Previously, we have shown that chronic intermittent hypoxia (CIH) impairs baroreflex control of heart rate and augments aortic baroreceptor afferent function. In the present study, we examined whether CIH induces structural changes of aortic afferent axons and terminals. Young-adult Fischer 344 (F344, 4 months old) rats were exposed to room air (RA) or CIH for 35–45 days. After 14–24 days of exposure, they received tracer DiI injection into the left nodose ganglion to anterogradely label vagal afferent nerves. After surgery, animals were returned to their cages to continue RA or CIH exposure. Twenty-one days after DiI injection, the animals were sacrificed and the aortic arch was examined using confocal microscopy. In both RA and CIH rats, we found that DiI-labeled vagal afferent axons entered the wall of the aortic arch, then fanned out and branched into large receptive fields with numerous terminals (flower-sprays, end-nets and free endings). Vagal afferent axons projected much more to the anterior wall than to the posterior wall. In general, the flower-sprays, end-nets and free endings were widely and similarly distributed in the aortic arch of both groups. However, several salient differences between RA and CIH rats were found. Compared to RA control, CIH rats appeared to have larger vagal afferent receptive fields. The CIH rats had many abnormal flower-sprays, end-nets, and free endings which were intermingled and diffused into "bush-like" structures. However, the total number of flower-sprays was comparable ($P>0.05$). Since there was a large variance of the size of flower-sprays, we only sampled the 10 largest flower-sprays from each animal. CIH substantially increased the size of large flower-sprays ($P<0.01$). Numerous free endings with enlarged varicosities were identified, resembling axonal sprouting structures. Taken together, our data indicate that CIH induces significant remodeling of afferent terminal structures in the aortic arch of F344 rats. We suggest that such an enlargement of vagal afferent terminals may contribute to altered aortic baroreceptor function following CIH.

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Abbreviations: ADN, aortic depressor nerve; CIH, chronic intermittent hypoxia; DiI, 1,1′-dioleyl-3,3,3′,3′-tetramethylindocarbocyanine methanesulfonate; F344, Fischer 344; HR, heart rate; RA, room air.

Key words: baroreflex, nodose ganglia, baroreceptor afferent, aortic arch, chronic intermittent hypoxia, sleep apnea.

Previously, we have demonstrated that baroreflex control of heart rate (HR) (baroreflex sensitivity) is impaired in F344 rats following chronic intermittent hypoxia (CIH) exposure (Gu et al., 2007; Lin et al., 2007). However, the neural mechanism underlying such baroreflex impairment is not well understood. Our ultimate goal is to systematically study CIH-induced structural remodeling and functional alteration of multiple neural components within the baroreflex circuitry. Since vagal afferent terminals in the aortic arch are baroreceptors and the first neural element within the baroreflex arc, we speculated that baroreflex impairment was likely associated with attenuation of aortic baroreceptor afferent function. Opposite to this assumption, we found that aortic depressor nerve (ADN) activity in response to arterial pressure increase is actually enhanced following CIH (Gu et al., 2007), suggesting that CIH augments aortic baroreceptor afferent function. It is likely that such a functional change may be associated with anatomical remodeling of ADN innervation of the aortic arch. Previously, we examined vagal afferent axons and terminals in the aortic arch and atria of Sprague–Dawley (SD) rats (Cheng et al., 1997a,b). We observed that vagal afferent axons innervated the walls of the aortic arch and atria with differentiated terminal structures (flower-sprays, end-nets, and free endings). In this study, we used anterograde tracing and confocal microscopy to examine whether CIH induces morphological changes of vagal afferent axons and terminals in the aortic arch.

EXPERIMENTAL PROCEDURES

F344 rats (4 months old, $n=6$/group) were used. Procedures were approved by the University of Central Florida Animal Care and Use Committee and followed the guidelines established by the National Institutes of Health. Efforts were made to minimize the suffering of the animals and the number of animals used.

Intermittent hypoxia exposure

Animals were housed in Plexiglass chambers ($30\times20\times20$ in^3; Oxycycler model A44XO, BioSpherix Instruments, Redfield, NY, USA) in a room with 12-h light/dark cycles (light from 6:00 AM to 6:00 PM). O_2 concentration in animal chambers was continuously measured by an O_2 analyzer and controlled through a computerized gas valve system. O_2 concentrations in the chambers were preprogrammed and automatically adjusted. Any deviation from

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the desired O_2 concentration was corrected by adding pure N_2 or O_2 through solenoid valves. Ambient CO_2 in the chambers was periodically monitored and maintained at 0.03% by adjusting overall chamber ventilation. Humidity was measured and maintained at 40–50%. Temperature was kept at 22–24 °C. The intermittent hypoxia (IH) profile consisted of alternating 21% (90 s) and 10% (90 s) O_2 every 6 min for 12 h during the light cycle and O_2 maintained at 21% for the night period, with an overall exposure duration of 35–50 days. The room air (RA) control animals were housed in room air under the same conditions as IH-exposed animals, except that the concentration of O_2 was maintained at 21% throughout the duration of exposure.

Tracer injections

Fifteen to 24 days after exposure, animals were anesthetized with Nembutal (50 mg/kg i.p.; Hospira, Inc., Lake Forest, IL, USA). A midline incision was made along the neck, and ventral neck muscles were gently separated by blunt dissection to expose the nodose ganglion medial to the internal carotid artery (Cheng et al., 1997a,b). To label vagal afferent projections to the aortic arch, multiple injections of tracer DiI (total 0.5 μl, 1,1′-dioleyl-3,3,3′,3′-tetramethylindocarbocyanine methanesulfonate, catalogue number D-3886, Molecular Probes, OR, USA) were administered to the left nodose ganglion by means of a micropipette connected to a picospritzer (Fig. 1). After completion of all injections, the surgical wound was closed with sutures and the animal was returned to its cage for continuation of RA or IH exposure.

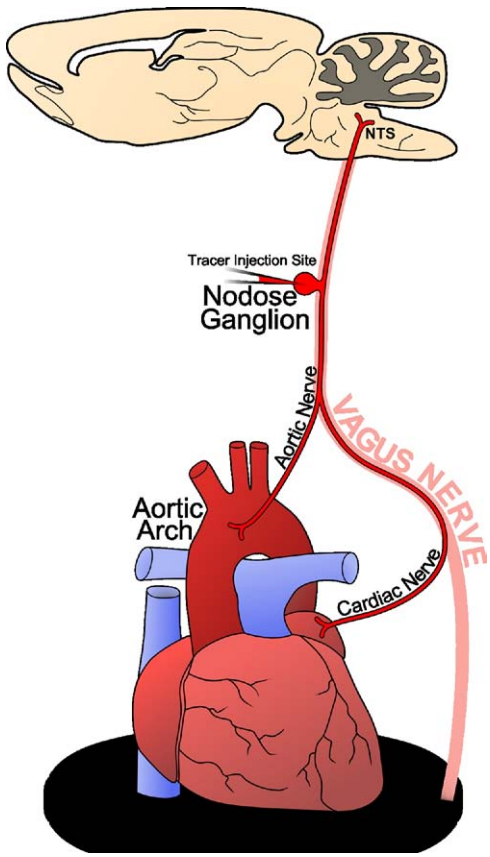

Fig. 1. Schematic drawing of tracer DiI injection into the left nodose ganglion to anterogradely label ADN axons and terminals in the aortic arch. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

Tissue processing

After a survival period of 21 days, each animal was anesthetized with an overdose of Nembutal (100 mg/kg i.p.), and perfused through the heart with warm 0.9% saline (300 ml) and phosphate-buffered (pH 7.4, 100 ml) 10% formalin (500 ml). Using a surgical microscope, the aortic arch, including the bifurcation regions of common carotid and subclavian arteries, and its surrounding tissues were identified. This tissue was removed immediately after perfusion and stored in 10% formalin for at least 2 hours. The removed aortic arch was cut longitudinally through the lumen into halves. Specimens were dehydrated and cleared in a series of ascending concentrations of glycerin (70, 90, 2×100%; 4 h each). The tissue was mounted on slides in 100% glycerin to which 5% N-propylgallate had been added as an anti-fade agent (Cheng et al., 1997a,b).

Data acquisition and analysis

Specimens were first screened with an epifluorescence microscope (Nikon Eclipse 80i). When DiI-labeled nerve fibers and endings were found at 200× magnification, their locations were noted for later laser confocal microscopic analysis and reconstruction. Using the confocal microscope, we scanned and documented each nerve bundle and terminal structure as a series of optical sections (Z-step: 1 μm; objective: 20×, zoom: 2×) for later analysis. As previously demonstrated (Cheng et al., 1997b), vagal axon fibers and their flower-sprays and end-net terminal structures were easily identifiable. Since the size of flower-sprays varied substantially, we only selected the 10 largest flower-sprays from each animal to quantify the size of flower-sprays. In this way, we could ensure that only flower-sprays of large axons were quantified and compared. We used confocal software to calculate the area of each flower-spray, and averaged the areas of the 10 largest flower-sprays for each rat. To quantify the area of a flower-spray, the flower-sprays were first scanned using the confocal microscope. The brightness and contrast for the scanning were adjusted to cover the entire range of gray intensity values: 0–255 with a few darkest (gray level 0) and saturation (gray level 255) pixels. The area of a flower-spray was measured as the total pixel area above a pre-selected threshold (consistent for all flower-sprays) measured inside the terminal arborization region (manually outlined as seen in Fig. 8A and B) in the projected image. In order to avoid the effects due to CIH-induced size changes of the aorta, we measured the circumference of the aorta at the bifurcation regions of common carotid and subclavian arteries in RA and CIH rats. Then, we normalized the area of each flower-spray in CIH rats by the square of the average ratio of the aortic arch circumference of RA/CIH rats (86%). Data are expressed as mean±SE. Differences of the size of axons and terminals between groups were determined using Student *t*-tests. $P<0.05$ was considered as statistically significant.

RESULTS

DiI-labeled vagal afferent axons and terminals in the aortic wall in RA rats: an overview

In confocal scans of the aortic arch and its surrounding tissue in RA rats, DiI injection into the nodose ganglion extensively labeled vagal afferent axons. In each animal, DiI-labeled vagal afferent axon bundles entered both (an-