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ATTENUATION OF HEART RATE CONTROL AND NEURAL DEGENERATION IN NUCLEUS AMBIGUUS FOLLOWING CHRONIC INTERMITTENT HYPOXIA IN YOUNG ADULT FISCHER 344 RATS

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Abstract—Chronic intermittent hypoxia (CIH) attenuates baroreflex control of heart rate (HR). In this study, we assessed whether CIH exposure reduced nucleus ambiguus (NA) control of HR and induced neural degeneration in the NA. Fischer 344 (age: 3–4 months) rats were exposed to either room air (RA: normoxia) or intermittent hypoxia for 35–50 days. At the end of these exposures, animals were anesthetized with pentobarbital. HR responses to arterial blood pressure (AP) changes induced by phenylephrine (PE) and sodium nitroprusside (SNP) were measured. In another set of rats, HR and AP responses to L-glutamate (L-Glu) microinjections (10 mM, 20 nl) into the left NA and electrical stimulation of the left cervical vagus nerve at 1–30 Hz (0.5 mA, 1 ms) for 20 s were measured. Brainstem slices at the level of −800, −400, 0, +400, +800 μm relative to the obex were processed in additional rats using Nissl staining. The NA was identified by retrogradely labeling vagal motoneurons using the tracer tetramethylrhodamine dextran (TMR-D) which was injected into the ipsilateral nodose ganglion. We found that CIH significantly 1) reduced the baroreflex control of HR (slope RA: −1.2±0.2 bpm/mmHg; CIH −0.5±0.1 bpm/mmHg; $P<0.05$); 2) attenuated the HR responses to L-Glu injections into the NA [HR: −280±15 (RA) vs. −235±16 (CIH) beats/min; $P<0.05$]; 3) augmented the HR responses to electrical stimulation of the vagus ($P<0.05$); 4) induced a significant cellular loss in the NA region ($P<0.05$). Thus, CIH induces a cell loss in the NA region which may contribute to attenuation of baroreflex sensitivity and NA control of HR following CIH.

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Abbreviations: AP, arterial blood pressure; CIH, chronic intermittent hypoxia; CVLM, caudal ventrolateral medulla; DmnX, dorsal motor nucleus of the vagus; HR, heart rate; L-Glu, L-glutamate; MAP, mean arterial pressure; NA, nucleus ambiguus; NTS, nucleus of the solitary tract; OSA, obstructive sleep apnea; PE, phenylephrine; RA, room air; RVL, rostroventrolateral nucleus; RVLM, rostral ventrolateral medulla; SNP, sodium nitroprusside; TMR-D, tetramethylrhodamine dextran; VRG, ventral respiratory group; ΔHR, changes in heart rate; ΔMAP, changes in mean arterial pressure.

Key words: baroreflex, nucleus ambiguus, parasympathetic, cardiac ganglia, heart, intermittent hypoxia.

Chronic intermittent hypoxia (CIH) during sleep has been used as an experimental model for obstructive sleep apnea (OSA) (D. Gozal et al., 2001, 2003). Similar to patients with OSA (Bonsignore and Mancia, 1997, 2006; Bradley et al., 2003), baroreflex control of heart rate (HR) (baroreflex sensitivity) is significantly reduced following CIH in C57BL/6J mice (Lin et al., 2007a). The reduction of baroreflex sensitivity could reflect changes within multiple sites in the brain–heart neural circuitry. Recently, we showed that CIH significantly reduces central mediation of baroreflex control of HR, but augments aortic barorecepter afferent function and vagal efferent control of HR (Gu et al., 2007). These findings suggested that the central components in the baroreflex circuitry [e.g. baroreceptor afferent terminals in the nucleus of the solitary tract (NTS), neurons in NTS, vagal motoneurons, and their network connections] contribute to the attenuation of baroreflex sensitivity caused by CIH, whereas the activity of peripheral vagal nerves is up-regulated to compensate for the functional reduction in central components. Both the nucleus ambiguus (NA) and dorsal motor nucleus of the vagus (DmnX) project strongly to cardiac ganglia in rats (Cheng et al., 1999, 2004a; Cheng and Powley, 2000), indicating that they are both involved in HR control. However, lesions to the NA abolished the baroreflex sensitivity, while lesions of the DmnX did not have any effect, implying that the NA plays a critical role in baroreflex control of HR (Cheng et al., 2002, 2004b; Jones et al., 1998). In this study, we focused on the functional and anatomical changes of the NA neurons following CIH exposure. Since baroreflex sensitivity is significantly reduced, we hypothesized that significant functional changes and anatomical degeneration of motoneurons in the NA may have developed following CIH.

EXPERIMENTAL PROCEDURES

Fischer 344 (F344; 3–4 months) rats were used. Procedures were approved by the University of Central Florida Animal Care and Use Committee and followed the guidelines established by the NIH. Efforts were made to minimize the number of animals used and reduce animal suffering. One of our long-term goals is to study whether CIH induces more severe damage to the baroreflex circuitry in aged rats than in young rats (Yan et al., 2006). Since F344 rat is one of the most commonly used animal model for study of aging, this rat strain was therefore selected.

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Intermittent hypoxia exposure

Animals (age: 3–4 months) were housed in Plexiglas chambers (30×20×20 in^3; Oxycycler model A44XO; BioSpherix Instruments, Redfield, NY, USA) in a room with 12-h light/dark cycles (6:00 am to 6:00 pm). O_2 concentration in the chambers was continuously measured by an O_2 analyzer and controlled by a computerized system through a gas valve outlet. O_2 concentration in chambers was programmed and adjusted automatically. Any deviation from the desired concentration was corrected by adding pure N_2 or O_2 through solenoid valves. Ambient CO_2 in the chamber was periodically monitored and maintained at 0.03% by adjusting overall chamber basal 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 during the light period and maintained at 21% O_2 during the dark period. The overall exposure duration was 35–50 days. The room air (RA) control animals were housed in RA exactly under the same conditions as animals in the Oxycycler chambers, except for the concentration of O_2 which was continuously maintained at 21% (D. Gozal et al., 2001; Gu et al., 2007; Lin et al., 2007a).

Analysis of baroreflex control of HR (baroreflex sensitivity). Rats were initially anesthetized with sodium pentobarbital (50 mg/kg i.p.). The trachea was cannulated and the rats breathed spontaneously. Body temperature was maintained at 37±1 °C with a homeostatic blanket (Harvard Apparatus, Holliston, MA, USA) and a rectal probe. Polyethylene catheters (PE-50) were placed in a femoral artery to monitor mean arterial pressure (MAP) and in a vein to infuse phenylephrine (PE) or sodium nitroprusside (SNP). The hind paw pinch withdrawal reflexes were used to check for the depth of anesthesia. Supplemental doses of sodium pentobarbital (5 mg/kg) were given as needed. MAP was measured by pressure transducers and amplifiers (model P23db; Statham Laboratories, Hato Rey, Puerto Rico, and Buxco with preamplifier model MAX II 2270; Buxco Electronics, Troy, NY, USA). HR was measured by a tachograph (model 7P4H; Grass Instrument, Quincy, MA, USA) triggered from the arterial pressure pulse. MAP and HR were recorded by an oscillograph (model 7 Polygraph, Grass Instrument, Quincy, MA, USA), and beat-to-beat analog signals were digitized and recorded for subsequent offline analyses.

SNP and PE (Sigma, St. Louis, MO, USA) were freshly prepared, diluted in 0.9% NaCl, and infused by a syringe pump (Sp101i; Stoelting, Wood Dale, IL, USA) for 1–2 min (6–12 μg/min) to achieve a HR plateau. Infusions were repeated three times to obtain an average response for each animal. At least 20 min were allowed between infusions to allow MAP and HR to return to baseline. Baseline MAP and HR were measured for a 20-s interval before infusion. The HR response was measured at the HR plateau, and the MAP response was measured at the point corresponding to the beginning of the HR plateau. Data were plotted as changes in heart rate (ΔHR) versus changes in MAP (ΔMAP) in response to PE or SNP. Because the HR–MAP relationship can be approximated by a sigmoidal logistic curve (Kent et al., 1972), we applied regression analysis to the linear portion of the responses for each animal to calculate the slope of the regression line, which was used as an index for baroreflex sensitivity (Soukhova-O'Hare et al., 2006).

Cardiovascular responses to microinjection of L-glutamate (L-Glu) into the NA. Animals were initially anesthetized with sodium pentobarbital (50 mg/kg i.p.) and artificially ventilated with oxygen-enriched RA (Harvard Rodent Ventilator Model 683; respiratory rate: 70–80 /min, tidal volume 0.5 ml/100 g, Holliston, MA, USA). The body temperature was maintained at 37±1 °C with a homeostatic blanket and a rectal probe (Harvard Apparatus). Anesthetic and pharmacological drugs were delivered through separate catheters in the left and right femoral veins. The surgical procedure to expose the brainstem was identical to that previously described (Cheng and Powley, 2000; Cheng et al., 2004a,b). Briefly, animals were placed in a stereotaxic instrument equipped with a head holder adapted to permit the neck to be sharply flexed. A dorsal incision was made over neck muscles, which were retracted to expose the atlantooccipital membrane. The membrane was opened with an incision, exposing the cisterna magna and the dorsal medulla. The caudal end of the area postrema was used as a rostrocaudal reference for stereotaxic coordinates. Using a multi-injection system (MDI, PM8000, South Plainfield, NJ, USA), L-Glu 10 mM (20 nl) was first determined as the saturation dose and hence was used in the remainder of the experiments. L-Glu (10 mM, 20 nl) was microinjected into the left NA at the level of the area postrema where repeated injections reliably induced maximal bradycardia and hypotension. The maximal changes in HR and MAP relative to the prestimulus baseline values were measured. The injection sites for the maximal responses were experimentally determined by injecting the different sites along and around the NA at the level of the area postrema. The volume delivered was monitored by the displacement of the meniscus in the pipette through a microscope fitted with an eyepiece graticule. Saline was injected at the same site to provide a vehicle control. Injection sites were comparable because similar coordinates were used in all animals. Previously, we have demonstrated that injection sites were consistently in and around the NA region (Cheng and Powley, 2000; Cheng et al., 2004a,b). In this study, after completion of the experiment in four animals, we used a multichannel injector to inject the tracer DiI into the same location where the maximum responses were evoked by L-Glu injections to mark the injection site.

Cardiovascular responses to electrical stimulation of the vagal nerve. L-Glu injection into the NA may activate vagal cardiac motoneurons through glutamate receptors. In turn, vagal cardiac motoneurons control the heart through vagal efferent innervation of cardiac ganglia. In order to evaluate the separate effects of vagal cardiac motoneurons and vagal cardiac efferent input to the heart, we electrically stimulated the left vagal nerve. The left vagus was transected distal to the nodose ganglion. The vagal nerve distal to the cut was stimulated by a pair of bipolar electrodes with the conventional arrangement, i.e. the cathode was near the heart. Square pulses were generated using a stimulator (S48K, Grass Instruments) at frequencies of 1–30 Hz (20 s) and delivered through a stimulus isolation unit (PSIU6, Grass Instrument) which provided the nerve with constant current (0.5 mA, 1 ms) during stimulation. Before the next stimulation, a 5 min period was allotted for the HR and MAP responses to return and stabilize at the pre-stimulus baseline values before the next stimulation. HR and MAP baseline values were determined from the average values 60 s prior to stimulation. The maximum responses of the HR and MAP were calculated from the 20-s stimulation period. In addition, the time from the stimulus onset to the point of 50% of the peak HR response was measured. HR and MAP returned to the pre-stimulus baseline values shortly after stimulation. Response curves of the HR and MAP were plotted against stimulus frequency.

Labeling of vagal motoneurons with tetramethylrhodamine dextran (TMR-D)

Identification of the NA region in the brainstem. We injected TMR-D (35 μg in 0.5 μl distilled water, molecular weight of 3000, catalog number D-3308, Molecular Probes, Carlsbad, CA, USA) into the nodose ganglion to label vagal motoneurons in the NA. Twelve animals (n=6 for left and 6 for right) of the same age and size were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A midline incision was made along the neck, and the ventral neck muscles were gently separated by blunt dissection to expose the nodose ganglion (Cheng et al., 1997, 2004b). Multiple injections of