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Research article

Does acute heat stress differentially-modulate expression of ionotropic neurotransmitter receptors in the RVLM of young and aged F344 rats?

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ABSTRACT

The rostral ventral lateral medulla (RVLM) is a brainstem area that plays a role in regulating numerous physiological systems, especially their responsiveness to acute stress. Aging affects the responsiveness of RVLM neural circuits to acute stress. Based on the relationship between ionotropic neurotransmitter receptors in the RVLM and the physiological functions mediated via activation of these receptors, we hypothesized that in response to acute heat stress the expression of ionotropic neurotransmitter receptors in the RVLM of aged rats would be characterized by upregulation of inhibitory subunits and downregulation of excitatory subunits. The goal of the present study was to determine the effect of acute heating on the gene expression profile of RVLM inhibitory ($GABA_A$ and Glycine) and excitatory (NMDA and AMPA) ionotropic neurotransmitter receptor subunits in young and aged F344 rats. RVLM tissue punches from young and aged F344 rats were analyzed using TaqMan qPCR and immunoblotting. When compared to age-matched controls, heat stress increased the gene expression of RVLM inhibitory receptor subunits in aged (*Gabra1*, *Gabra2*, *Gabra5*, *Glra1*) and young (*Gabra1*) F344 rats at mRNA level, with little change in the expression of RVLM excitatory receptor subunits. Significant age x heat interaction effects were observed with increased expression of *Gabra2* and *Gabrb1* inhibitory receptor subunits and decreased expression of *Gria1* and *Gria2* excitatory receptor subunits in the RVLM of aged F344 rats, with the most marked change observed with the *Gabra2* subunit, which was validated by immunoblotting. These findings demonstrate that in response to acute heat stress there is enhanced expression of inhibitory ionotropic receptor subunits in aged compared to young rats, supporting the idea that advanced age may alter RVLM responsivity by affecting the molecular substrate of ionotropic receptors.

1. Introduction

The rostral ventral lateral medulla (RVLM) is an area of the brainstem that contains a diverse array of neurons involved in the regulation of vital physiological processes including those serving respiration, blood pressure, metabolic sensing, and numerous visceral organ functions [3,11,22,23,35,38]. The activity level of RVLM neurons results from a balance of excitatory and inhibitory neural states, with NMDA and AMPA ionotropic receptors involved in glutamatergic-mediated excitation, and $GABA_A$ and glycine ionotropic receptors playing key roles in neuronal inhibition [1,10,20,39]. For example, RVLM microinjections of glutamate (excitatory amino acid receptor agonist) and bicuculline (GABA receptor antagonist) increase whereas RVLM microinjections of muscimol ($GABA_A$ receptor agonist) decrease blood pressure and sympathetic nerve activity [9,14,19]. Both excitatory and inhibitory ionotropic neurotransmitter receptors in the central nervous system are characterized by complexes composed of multiple subunits that allow for distinct composition profiles and the formation of heteromeric ion channels. Specifically, $GABA_A$ receptor pentamers (pool of 19 different subunits), glycine receptor tetramers (α1-4 and β subunits), glutamatergic NMDA receptor tetramers (*Grin1* and *Grin2a*, *Grin2b*, *Grin2c* and *Grin2d* subunits), and AMPA receptor tetramers (*Gria1*, *Gria2*, *Gria3* and *Gria4* subunits) are assembled from and composed of multiple subunits [5,24,27,37].

Based on the relationship between the structural complexity of ionotropic receptors and the physiological functions mediated via activation of these receptors, it is likely that changes in physiological regulation associated with disease states or advancing age are related to modifications in the expression profiles of key RVLM receptor systems or receptor subunits. We are interested in understanding how aging affects the regulation of RVLM-related systems, with a focus on determining the effect of advancing age on the responsiveness of RVLM neural circuits to acute stress. Previous studies have employed acute heating as an environmental stressor because increased internal body

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temperature affects numerous physiological functions associated with RVLM neural circuits, including; respiration, arterial blood pressure, and sympathetic nerve outflow [4,7,15,25]. Heating-induced increases in sympathetic nerve outflow and arterial blood pressure are attenuated in aged compared with young F344 rats [16,17], suggesting a role for age-related changes in the central regulation of physiological function in response to increased internal body temperature. However, it remains unknown if the molecular substrate of RVLM ionotropic receptor subunits demonstrates age-dependent changes in response to acute heat stress.

The goal of the present study was to determine the effect of acute increases in internal body temperature (Tc) on the transcriptomic profile of RVLM ionotropic receptor subunits in young and aged F344 rats. We tested the hypothesis that in response to acute heat stress the expression of ionotropic neurotransmitter receptors in the RVLM of aged rats would be characterized by upregulation of inhibitory subunits and downregulation of excitatory subunits. Because of the limited understanding regarding RVLM sub-regional anatomy and the difficulty ensuring that any specific subsystem with the RVLM can be reliably sampled, the present experimental approach was focused on in the global RVLM. We determined relative gene expression profiles of RVLM ionotropic inhibitory ($GABA_A$ and Glycine) and excitatory (NMDA and AMPA) receptor subunits at mRNA level in brain tissue containing RVLM micropunches in young (3–4 months) and aged (22–24 months) F344 rats using the TaqMan qPCR approach, along with select protein expression completed using immunoblotting. Relative gene and protein expression profiles were determined after Tc had been increased to 41.5 °C and compared with non-heated (Tc maintained at 38 °C) F344 rats.

2. Methods

All procedures and protocols were approved by the Institutional Animal Care and Use Committee and were completed in accordance with the American Physiological Society's guidelines for research involving animals.

2.1. General procedures

Experiments were completed in young adult (3–4 months; n = 18, 300 ± 6 g) and aged (22–24 months; n = 18, 423 ± 15 g) male Fischer 344 rats (Charles River Laboratories, contracted with National Institute on Aging). F344 rats are a strain of rats provided by the National Institute on Aging for studies focused on aging research, and are widely used in this research domain [26]. Many studies that have employed direct sympathetic nerve recordings and central microinjections (including our previous studies) to determine the effects of advancing age on sympathetic nervous system regulation have utilized F344 rats as the preferred rodent model [8,13,19].

Anesthesia was induced by isoflurane (5%; Butler Animal Science) and maintained during surgical procedures using isoflurane (1.5%–2.5%), α-chloralose (50 mg/kg, iv; Sigma), and urethane (400 mg/kg, ip; Sigma) [18]. Catheters were placed in the femoral vein for the intravenous administration of maintenance doses of α-chloralose (35–45 mg/kg/hr) and in the femoral artery for measurement of arterial blood pressure and heart rate. Isoflurane anesthesia was discontinued following surgical procedures. Tc was measured with a thermistor probe inserted approximately 5–6 cm into the colon and was kept at 38 °C during surgical interventions by a temperature-controlled table. The adequacy of anesthesia was indicated by the absence of a somatic response to mechanical stimulation of the tail or hind limb.

2.2. Heat stress and analytical protocols

Before the initiation of experimental protocols, anesthetized rats were allowed to stabilize for 60 min. In non-heated control rats Tc was maintained at 38 °C for 60 min following completion of the stabilization period. In heated rats, following completion of the stabilization period, Tc was increased from 38 °C to 41.5 °C over the course of 30 min at an approximate rate of 0.1 °C/min using a heat lamp and a homeothermic blanket control unit (Harvard Apparatus), and maintained at 41.5 °C Tc for an additional 25 min. At the end of experiments rats were euthanized via an overdose of methohexital sodium (Brevital, 150 mg/kg, iv; JHP Pharmaceutical). Brains were collected and snap-frozen in liquid nitrogen. All brains were stored at 80 °C until use.

2.3. Brain sectioning and micropunching

Rats were divided into two groups, the first group of young (n = 10) and aged (n = 10) F344 rats was used for gene expression studies, and with second group of young (n = 8) and aged (n = 8) was used for immunoblotting studies. The brains collected from young and aged rats were subjected to brain sectioning and RVLM micropunching protocols that were performed as described in our previous manuscript [2]. The stereotaxic coordinate system along with its photographic documentation of histologically stained hindbrain sections of the rat brain atlas by Paxinos and Watson [31], were used as references to locate and identify the hindbrain region containing the RVLM. This was achieved through two approaches; first, a priori: before the sampling, inferred stereotaxic space was used to perform calibrated sectioning based on stereotaxic boundaries of the RVLM (Bregma coordinate). Second, a posteriori: after the sampling, histological analysis was performed to identify cytoarchitectonically demarcated fiducial structures that marked the rostral pole of the RVLM and with successive tissue sections RVLM was located, as documented histologically by the photomicrographs within the atlas.

2.3.1. A priori approach

Coronal sections of the hindbrain were made through the rostrocaudal extent of the RVLM using a cryostat (Leica CM3050S, Leica Biosystems Inc, IL). Following the identification of lobule 2 of the cerebellar vermis (abbreviated '2Cb'; Figure 103 of Paxinos and Watson [31]), the rostral fiducial that was used to mark the beginning of calibrated sectioning; ninety 40μm-thick serial sections (~3.6 mm) were obtained and discarded. Immediately after the remaining tissue blockface was used to collect two serial 200μm-thick coronal sections (beginning at an inferred distance from Bregma of –12.0 mm, or approximately at Figure 133, and extending to –12.4 mm from Bregma, or approximately at the level shown in Figure 136 of Paxinos and Watson [31]). Once the tissue sections were obtained, the RVLM-containing area was micropunched bilaterally with a Harris micropunch (inner diameter = 1 mm) on a frozen block maintained at –10 °C to − 20 °C, using a modification of the Palkovits micro-punch technique [28,29]. All four tissue punches from each brain were pooled into an RNase-free centrifuge tube and stored at − 80 °C until further use. The micropunch needle and the freezing block were cleaned with 70% alcohol followed by application of RNaseZap (Ambion, USA), to avoid cross-contamination and RNA degradation.

2.3.2. A posteriori approach

To identify the RVLM sample area, a fiducial analysis was performed as discussed in our previous manuscript [2]. The micropunched sections (200 μm) were stained with cresyl violet and examined for the location of the punched area relative to other landmarks such as, the fourth ventricle (4 V), spinal trigeminal tract (sp5), inferior olive (IO) and pyramidal tract (py), and dorsal compact formation of the nucleus ambiguus (AmbC). Representative images of cresyl violet stained micropunched sections from young and aged brains are presented in Fig. 1A and B, respectively.