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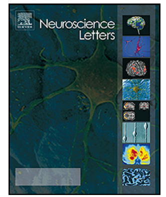

Research article

Disinhibition of neurons of the nucleus of solitary tract that project to the superior salivatory nucleus causes choroidal vasodilation: Implications for mechanisms underlying choroidal baroregulation

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HIGHLIGHTS

- NTS neurons controlling choroidal blood flow receive dense inhibitory input.
- Disinhibition of NTS neurons causes increases in choroidal blood flow.
- Disinhibition of NTS during low blood pressure may drive choroidal vasodilation.
- Sign inversion of a low blood pressure signal may drive choroidal baroregulation.

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ABSTRACT

Preganglionic neurons in the superior salivatory nucleus (SSN) that mediate parasympathetic vasodilation of choroidal blood vessels receive a major excitatory input from the baroresponsive part of the nucleus of the solitary tract (NTS). This input appears likely to mediate choroidal vasodilation during systemic hypotension, which prevents decreases in choroidal blood flow (ChBF) due to reduced perfusion pressure. It is uncertain, however, how low blood pressure signals to NTS from the aortic depressor nerve (ADN), which fires at a low rate during systemic hypotension, could yield increased firing in the NTS output to SSN. The simplest hypothesis is that SSN-projecting NTS neurons are under the inhibitory control of ADN-receptive GABAergic NTS neurons. As part of evaluating this hypothesis, we assessed if SSN-projecting NTS neurons, in fact, receive prominent inhibitory input and if blocking GABAergic modulation of them increases ChBF. We found that SSN-projecting NTS neuronal perikarya identified by retrograde labeling are densely coated with GABAergic terminals, but lightly coated with excitatory terminals. We also found that, infusion of the GABA-A receptor antagonist GABAzine into NTS increased ChBF. Our results are consistent with the possibility that low blood pressure signals from the ADN produce vasodilation in choroid by causing diminished activity in ADN-receptive NTS neurons that tonically suppress SSN-projecting NTS neurons.

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1. Introduction

Although ChBF was once thought to passively follow changes in perfusion pressure, many studies have now shown that ChBF remains stable over a systemic blood pressure range of about 35% above and below basal levels, with neural mechanisms involved in the compensation [17,23,33,35]. When blood pressure is low, choroidal vessels dilate to maintain blood flow to the eye, and when blood pressure is high, choroidal vessels constrict, preventing overperfusion and edema. Neurogenic choroidal vasodilation is mediated by parasympathetic innervation, and neurogenic choroidal vasoconstriction is accomplished by sympathetic

Abbreviations: Ach, acetylcholine; ADN, aortic depressor nerve; ABP, arterial blood pressure; ChBF, choroidal blood flow; ChBVol, choroidal blood volume; ChBVel, choroidal blood velocity; CVLM, caudal ventrolateral medulla; Glu, glutamate; GAD, glutamic acid decarboxylase; NO, nitric oxide; NOS, nitric oxide synthase; NTS, nucleus of solitary tract; RVLM, rostral ventrolateral medulla; SSN, superior salivatory nucleus; VIP, vasoactive intestinal polypeptide; VGLUT2, vesicular glutamate transporter 2.

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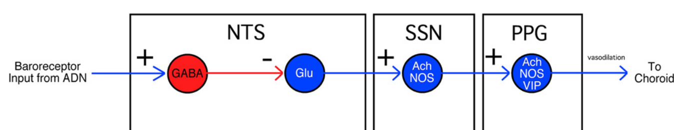

Fig. 1. Schematic illustrating the hypothetical circuit by which baroreceptive input to the NTS may mediate disinhibitory control of choroidal vasodilation via the SSN.

innervation. The vasodilation is mediated by the pterygopalatine ganglion (PPG), which innervates orbital vessels feeding the choroidal vessels, as well as choroidal vessels themselves [6,7,16,17,18,36,42], and employs the vasodilators nitric oxide (NO) and vasoactive intestinal polypeptide (VIP).

The preganglionic neurons regulating the PPG reside in the superior salivatory nucleus (SSN), the autonomic component of the facial motor complex [7], and SSN activation increases ChBF [42]. In prior studies in rats, we found that a major input to choroidal SSN arises from the nucleus of the solitary tract (NTS) [18,19]. The NTS is organized into viscerotopic subdivisions that receive input selectively from the tongue, vasculature, and thoracic and abdominal viscera [22,30,32]. Cardiovascular afferents predominantly project to the ipsilateral interstitial and dorsal NTS subnuclei at, and anterior to, the level of the obex [4,50]. Our studies have demonstrated that the part of NTS receiving and/or responsive to baroreceptor information is the source of a projection directly to the ChBF-control neurons of the SSN [18]. Since NTS input to SSN is excitatory [1,2,18] and NTS activation increases ChBF [20], the NTS input to SSN may mediate choroidal vasodilation during systemic hypotension. It is unclear, however, how low blood pressure signals to NTS from the aortic depressor nerve (ADN), which has excitatory input to NTS [21] and fires at a low rate during systemic hypotension [49], could yield increased firing in the output of NTS to SSN, and ultimately to choroidal vasodilation. One possibility is that SSN-projecting NTS neurons receive innervation from inhibitory ADN-receptive NTS neurons (Fig. 1). During normal or high systemic blood pressure, the SSN-projecting NTS neurons would be inhibited by these ADN-receptive NTS neurons. When blood pressure is low, however, the ADN-receptive inhibitory NTS neurons would fire at a low rate, with the SSN-projecting NTS neurons then being disinhibited. Consistent with this possibility, GABAergic neurons are present in baroreceptive NTS [13], and the distribution of neurons enriched in GABA-A receptors in NTS overlaps the distribution of SSN-projecting NTS neurons [38,45,46]. In the present study, we provide both morphological and physiological evidence to support this framework for blood pressure-dependent control of ChBF.

2. Methods

2.1. Animal preparation

Adult male Sprague-Dawley (SD) rats (290g–380 g; from Harlan, Indianapolis, IN) were used in either neuroanatomical studies to characterize inhibitory versus excitatory inputs to SSN-projecting NTS neurons (n = 3), or in physiological studies to assess the effects of a GABA-A antagonist infused into NTS on ChBF (n = 5). All experiments were undertaken in compliance with the ARVO statement on the Use of Animals in Ophthalmic and Vision Research, and with NIH guidelines and institutional approval.

2.2. Retrograde tracer injection into SSN

To identify SSN-projecting neurons in NTS, we made injections of BDA3K into SSN by iontophoresis, as described previously [18,19]. Animals were allowed to survive 10-14 days, and were then anesthetized, and perfused transcardially with 150 – 200 ml sodium phosphate buffered saline (PBS, 0.85% sodium chloride dissolved in 0.01 M PB) followed by 400–500 ml 4% paraformaldehyde prepared in 0.1 M sodium phosphate buffer (pH7.4) with 0.1 M lysine and 0.01 M sodium periodate (PLP fixative). Brains were removed, cryoprotected at 4 °C in 20% sucrose – 10% glycerol – 0.138% sodium azide in 0.1 M sodium phosphate buffer, frozen, and sectioned at 40 μm in the transverse plane with a sliding microtome. Sections were stored at 4°C in a 0.02% sodium azide – 0.02% imidazole in 0.1 M PB until processed for detection of VGLUT2-containing and GAD67-containing terminals on the BDA3K-containing SSN-projecting NTS neurons, as described below. Note that VGLUT2 is enriched in baroreceptive afferents ending in NTS [21].

2.3. Multiple-label fluorescence immunohistochemistry

We used immunofluorescence multiple labeling to visualize VGLUT2+ and GAD65+ terminals and BDA3K+ neurons in NTS. The sections were rinsed and incubated in streptavidin Alexa-488 (1:200; Molecular Probes) for 24 h at 4 °C to label the BDA3K+ SSN-projecting neurons in NTS. The sections were then rinsed and incubated in mouse anti-GAD65 (Sigma-Alrdich, 1:200) and either guinea pig anti-VGLUT2 (Millipore, 1:1000) or rabbit anti-VGLUT2 (Sigma-Aldrich, 1:1000), for 24 h at 4 °C. The antibodies were diluted with 0.1 M PB – 0.8% Triton X-100. After rinsing, the tissue was incubated in donkey anti-mouse IgG-Alexa-594 (1:200; Molecular Probes), and either goat anti-guinea pig IgG-Alexa-647 or donkey anti-rabbit IgG-Alexa-647 (1:200; Molecular Probes), overnight at 4 °C. The sections were then mounted on gelatin-coated slides, air-dried, and cover-slipped in glycerol phosphate buffer (9:1). Sections were viewed and images captured using a Zeiss 710 confocal laser-scanning microscope.

2.4. Choroidal blood flow measurements

For ChBF studies, rats were anesthetized by ip injection of 0.1 ml/100 g of ketamine/xylazine (87/13 mg/kg), with supplemental doses (0.05-0.07 ml of ketamine/xylazine mixture) every 25–30 min. To measure systemic arterial blood pressure (ABP), the femoral artery was catheterized. A Digi-Med Blood Pressure Analyzer™ (BPA-100, Micro-Med, Inc., Louisville, KY) was used to measure ABP via a pressure transducer connected to the femoral artery cannula. The rats were then positioned in a stereotaxic device. A pulse oximeter on the tail or hind foot measured systemic blood oxygen saturation. Body temperature was maintained at 37 °C with a Harvard heating blanket and rectal thermoprobe.

To prepare the eye for transcleral measurement of ChBF, fascia overlying the superior pole of the right eye and the Harderian gland was removed. The tip of a laser Doppler probe connected to a LASERFLO BPM^2 blood perfusion monitor (Vasamedics; Eden Prairie, MN) was positioned 1–3 mm above the sclera with a micromanipulator. Aquasonic ultrasound gel (Parker Laboratories, Inc.; Fairfield, NJ) or 33% glycerol – 0.1 M sodium phosphate buffer (PB) was used in the interface between the probe tip and the sclera to prevent tissue drying during the experiment. Measurements of ChBF were made from the vascular bed beneath the sclera in the gap between the superior and medial rectus muscles. Our previous work has shown that relative ChBF can reliably be measured transclerally using Laser Doppler Flowmetry [11,12,13,33]. The LASERFLO BPM^2 data was exported to a ML880 PowerLab 16/30 data acquisition system (AD Instruments Inc., Colorado Springs, CO), and the digitized data were collected and analyzed using LabChart v7 Pro software (AD Instruments Inc). The data averaging time window of the BPM^2 was set at 0.3 s, with the sampling rate on PowerLab set at 200 samples/s. For placement of a micropipet in NTS, the skin over the skull was

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