Brainstem inhibitory signaling as a novel sex-dependent regulator of energy homeostatic parasympathetic regulation

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INTRODUCTION

Although detailed mechanism(s) responsible are still controversial, two brain regions, the hypothalamus and the dorsal vagal complex (DVC), play critical roles in central energy homeostatic modulation. Injection of nutrient signals (e.g. insulin or glucose) into these regions can alter overall blood glucose through vagal activity. The DVC is made up of the dorsal motor nucleus of the vagus (DMV), the nucleus tractus solitarius (NTS) and area postrema. The DMV contains the cell bodies of neurons responsible for parasympathetic motor output to subdiaphragmatic viscera making them a final, central modulation point in parasympathetic activity. Inhibitory, GABAergic neurotransmission contributes significantly to vagal motor neuron activity. Elevating glucose in the DVC elevates blood glucose and influences descending parasympathetic motor drive through activation of inhibitory, GABAergic currents in males. A small body of evidence suggests that $GABA_A$ receptor activity exhibits sexually dimorphic regulation. This regulation is estrous cycle-dependent and occurs even in brain regions with no direct role in reproduction. However, no work to date has examined sex differences in or estrous cycle regulation of DMV neurons. Since other energy homeostatic signaling mechanisms demonstrate sexual dimorphism, these relationships need to be elucidated. Therefore, the present study investigated if estrous cycle modulates GABAergic neurotransmission to DMV neurons.

METHODS

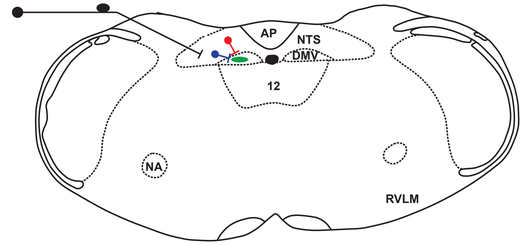

Figure 1: Illustration of the location of the DMV. Green oval represents the DMV neuron. Red and blue represent the GABAergic and glutamatergic innervations to the DMV from the nucleus tractus solitarius (NTS) respectively. Black represents the primary afferent. 12: hypoglossal nucleus; AP: area postrema; NA: nucleus ambiguus; RVLM:

IN VIVO GLUCOSE TOLERANCE

Experiments used 5-8 week old male and cycling female FVB mice, bred in-house. Estrus and diestrus was determined by vaginal smear. Anoestrous females were excluded.

When clear estrous states were present, mice were fasted for three hours and then adminstered 2mg/kg glucose through i.p. injection. Blood glucose was then montiored for 2 hours at regular intervals (15, 30, 60 and 120 min). To determine the role the parasympathetic nervous system plays in both basal, resting blood [glucose] and glucose tolerance. The muscarinic receptor antagonist, scopolamine methyl (0.5mg/kg; MSA) or vehicle saline was admintered 20 mins before glucose tolerance testing.

IN VITRO ELECTROPHYSIOLOGY:

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On the day of experimentation, mice were decapitated after an overdose with isoflurane. Brain tissue was collected and placed in ice-cold artificial cerebral spinal fluid (aCSF) bubbled with 95% O_2-5% CO_2. The composition of aCSF was as follows (in mM): 124 NaCl, 3 KCL, 26 $NaHCO_3$, 11 glucose, 1.3 $CaCl_2$, and 1.3 $MgCl_2$. Slices of 300 µm were made to include the DMV. Slices were transferred to a holding chamber and incubated in oxygenated aCSF at 32-34ºC.

Whole-cell patch-clamp recordings were performed using glass pipettes (2-5MΩ) filled with a solution containing the following (in mM): 130 Cs+ -gluconate, 1 NaCL, 5 EGTA, 10 HEPES, 1 $MgCl_2$, 1 $CaCl_2$, 3 CsOH and 2-3-ATP (2mM) at a pH or 7.3. Identified DMV cells were voltage-clamped at a holding potential of 0 mV. Kynurenic acid (KYN; 1mM) was added to perfusate to isolate GABAergic currents.

All recordings were low-pass filtered at 3kHz and acquired digitally at 20 kHz. Mini-analysis software was used to measure inhibitory postsynaptic current (IPSC) frequency, amplitude, and area.

RESULTS

Parasympathetic modulation of basal blood [glucose]

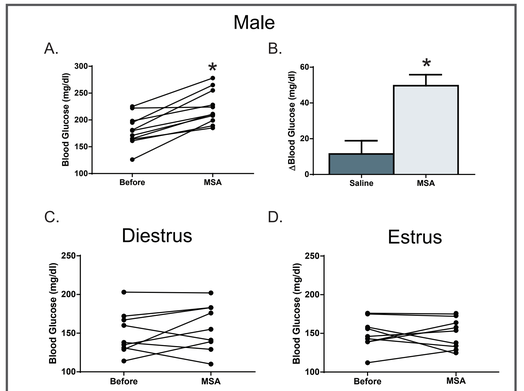

Figure 2: Sex difference in basal blood glucose regulation by the parasympathetic nervous system. A) Blood [glucose] before and after administeration of scopolamine methyl (MSA) in males (n=8). B) Mean and SEM of the change in blood [glucose] responses in (A) compared to the response after saline injection. C) Blood [glucose] before and after administeration of scopolamine methyl (MSA) in diestrous females (n=8). D) Blood [glucose] before and after administeration of scopolamine methyl (MSA) in estrous females (n=9). *significant difference

Parasympathetic modulation of glucose tolerance

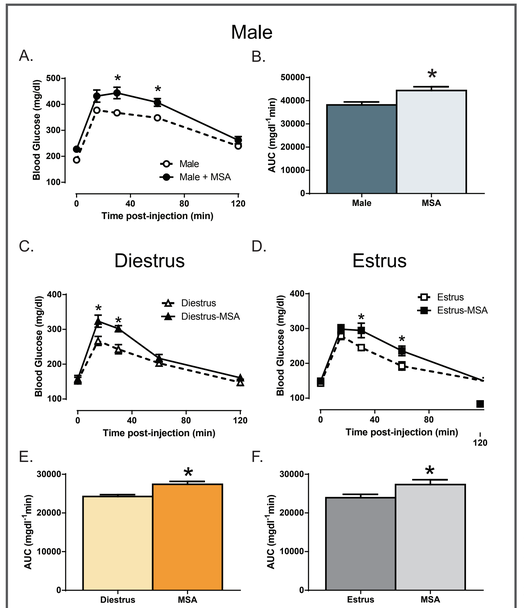

Figure 2: Estrous cycle regulation in the timing of parasympathetically mediated glucose tolerance. A) Glucose tolerance test in males before and after administeration of scopolamine methyl (MSA) (n=8). B) Area under the curve (AUC) of glucose tolerance curves in (A). C) Glucose tolerance test in diestrus females before and after administeration of scopolamine methyl (MSA) (n=8). D) Glucose tolerance test in estrus females before and after administeration of scopolamine methyl (MSA) (n=9). E) Area under the curve (AUC) of glucose tolerance curves in (C). F) Area under the curve (AUC) of glucose tolerance curves in (C).*significant difference

Mean sIPSC parameters are not modulated by estrous cycle

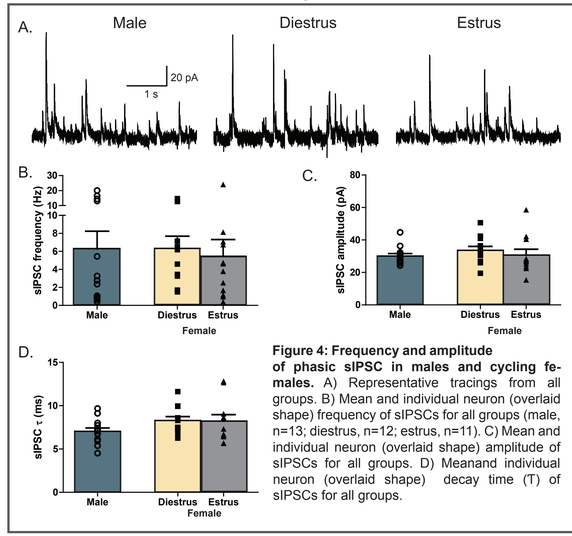

Figure 4: Frequency and amplitude of phasic sIPSC in males and cycling females. A) Representative tracings from all groups. B) Mean and individual neuron (overlaid shape) frequency of sIPSCs for all groups (male, n=13; diestrus, n=12; estrus, n=11). C) Mean and individual neuron (overlaid shape) amplitude of sIPSCs for all groups. D) Meanand individual neuron (overlaid shape) decay time (T) of sIPSCs for all groups.

Decreased phasic sIPSC variability during diestrus

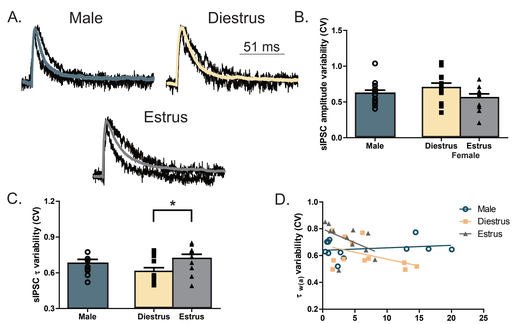

Figure 5: Variability of phasic sIPSCs' amplitude and decay time in males and cycling females. A) Representative tracings (scaled to amplitude) from one neuron per groups demonstrating decay time variability. B) Mean and individual neuron (overlaid shape) amplitude variability for all groups. (male, n=13; diestrus, n=12; estrus, n=11) C) Mean and individual neuron (overlaid shape) decay time variability for all groups. D) Correlation between decay time variability and frequency of sIPSCs. * significant difference (ANOVA with Tukey's)

Increased inhibitory tonic currents during diestrus

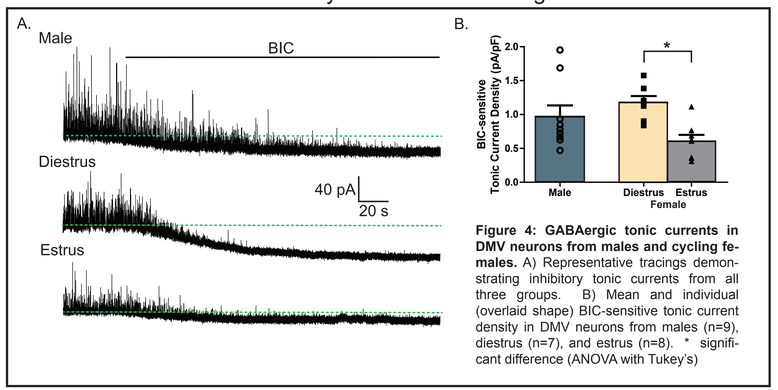

Figure 4: GABAergic tonic currents in DMV neurons from males and cycling females. A) Representative tracings demonstrating inhibitory tonic currents from all three groups. B) Mean and individual (overlaid shape) BIC-sensitive tonic current density in DMV neurons from males (n=9), diestrus (n=7), and estrus (n=8). * significant difference (ANOVA with Tukey's)

Decreased "THIP-Inducible" tonic currents during diestrus

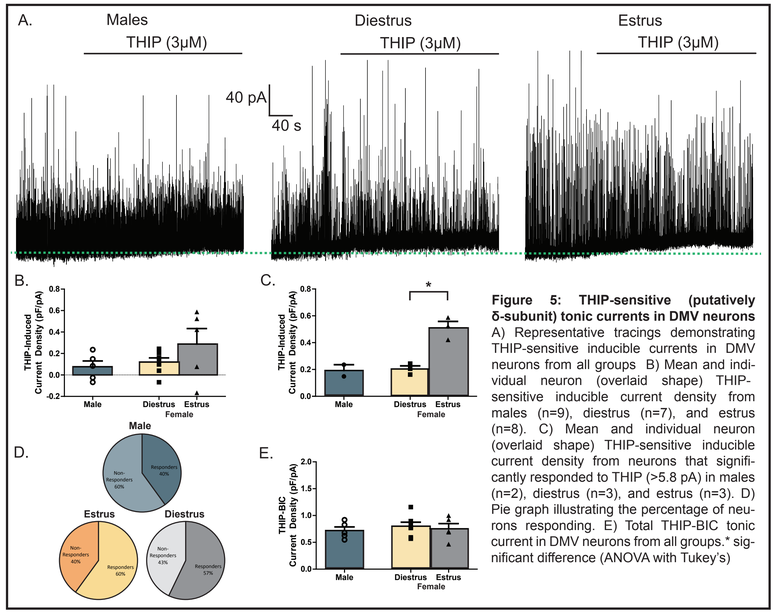

Figure 5: THIP-sensitive (putatively δ-subunit) tonic currents in DMV neurons A) Representative tracings demonstrating THIP-sensitive inducible currents in DMV neurons from all groups B) Mean and individual neuron (overlaid shape) THIP-sensitive inducible current density from males (n=9), diestrus (n=7), and estrus (n=8). C) Mean and individual neuron (overlaid shape) THIP-sensitive inducible current density from neurons that significantly responded to THIP (>5.8 pA) in males (n=2), diestrus (n=3), and estrus (n=3). D) Pie graph illustrating the percentage of neurons responding. E) Total THIP-BIC tonic current in DMV neurons from all groups.* significant difference (ANOVA with Tukey's)

CONCLUSIONS

1. In males, the parasympathetic nervous system contributes to both basal blood [glucose] and glucose tolerance. In females, regardless of estrous state, parasympathetic regulation is limited to glucose tolerance. However, during diestrous, glucose intolerance after parasympathetic blockade occurs largely within the first 30 minutes. Whereas in males and estrous females, the largest intolerance occurs during the 30 and 60 minutes, suggesting that the parasympathetic nervous system differentially regulates glucose metabolism based on estrous cycle.

2. Estrous cycle does not significantly modulate phasic sIPSC parameters. DMV neurons from females in diestrus have significantly less decay time variability than neurons from females in estrus. This is not likely mediated from an increased frequency of sIPSCs since frequency is not changed (figure 2) and frequency does not predict decay time variability. This is likely mediated through a reduced heterogeneity in postsynaptic receptor composition.

3. During diestrus, "resting" tonic $GABA_A$ receptor inhibitory currents in DMV neurons are significantly elevated. This elevated tonic current could be a result of either 1) elevated numbers of receptors peri-/extrasynaptic and/or 2) changes in receptor phosphorylation.

4. THIP-sensitive tonic currents in the DMV of estrous females are significantly elevated and demonstrate a significant "inducible" component. This elevated THIP-sensitivity increased the overall tonic current (THIP-BIC; figure 5) in estrous females, thereby "normalizing" them to the tonic currents demonstrated in DMV neurons from females in diestrus. It has been suggested that this "THIP-induced" current represents an unoccupied receptor population. Therefore, DMV neurons from estrus females may have a larger number of receptors that are not actively contributing to "resting" tonic currents, but are present in the membrane.

5. Overall, estrous cycle modulates both the contribution of parasympathetic regulation of glucose metabolism and inhibition in the neurons responsible for generating parasympathetic output,.Through a currently unknown mechanism(s), estrous cycle alters both phasic and tonic $GABA_A$ receptor activity. It was previously identified that experimentally-induced diabetes increases THIP-sensitivity in DMV neurons. Since estrous cycle also modulates similar neuronal physiology, females may have a unique susceptibility to diabetic perturbations of $GABA_A$ receptor activity in the DMV.

Supported By: R01 DK056132 to BNS and AHA 16SDG26590000 to CRB