Tonic GABA<sub>A</sub> Receptor-Mediated Inhibition in the Rat Dorsal Motor Nucleus of the Vagus

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Gao H, Smith BN. Tonic GABA<sub>A</sub> receptor-mediated inhibition in the rat dorsal motor nucleus of the vagus. J Neurophysiol 103: 904–914, 2010. First published December 16, 2009; doi:10.1152/jn.00511.2009. Type A γ-aminobutyric acid (GABA<sub>A</sub>) receptors expressed in the dorsal motor nucleus of vagus (DMV) critically regulate the activity of vagal motor neurons and, by inference, the gastrointestinal (GI) tract. Two types of GABA<sub>A</sub> receptor-mediated inhibition have been identified in the brain, represented by phasic (I<sub>phasic</sub>) and tonic (I<sub>tonic</sub>) inhibitory currents. The hypothesis that I<sub>tonic</sub> regulates neuron activity was tested in the DMV using whole cell patch-clamp recordings in transverse brain stem slices from rats. An I<sub>tonic</sub> was present in a subset of DMV neurons, which was determined to be mediated by different receptors than those mediating fast, synaptic currents. Preapplication of tetrodotoxin significantly decreased the resting I<sub>tonic</sub> amplitude in DMV neurons, suggesting that most of the current was due to action potential (AP)–dependent GABA release. Blocking GABA transport enhanced I<sub>tonic</sub> and multiple GABA transporters cooperated to regulate I<sub>tonic</sub>. The I<sub>tonic</sub> was composed of both a gabazine-insensitive component that was nearly saturated under basal conditions and a gabazine-sensitive component that was activated when extracellular GABA concentration was elevated. Perfusion of THIP (10 μM) significantly increased I<sub>tonic</sub> amplitude without increasing I<sub>phasic</sub> amplitude. The I<sub>tonic</sub> played a major role in determining the overall excitability of DMV neurons by contributing to resting membrane potential and AP frequency. Our results indicate that I<sub>tonic</sub> contributes to DMV neuron membrane potential and activity and is thus an important regulator of vagally mediated GI function.

INTRODUCTION

Type A γ-aminobutyric acid (GABA<sub>A</sub>) receptors are a family of pentameric membrane proteins gated by GABA, most of which are composed of α, β, and γ subunits, although 16 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π) have been identified in the mammalian CNS (Pirker et al. 2000; Rudolph et al. 2001; Sieghart 2000). Receptor subunit composition imparts specific kinetic and pharmacological properties to the receptors. Activation of GABA<sub>A</sub> receptors increases membrane permeability to chloride and their activation mediates most fast inhibitory postsynaptic currents (IPSCs) in the mammalian brain (Farrant and Nusser 2005; Rudolph et al. 2001; Vicini and Ortmis 2004). Inhibitory currents mediated by GABA<sub>A</sub> receptors in the brain can be divided into phasic (I<sub>phasic</sub>) and tonic (I<sub>tonic</sub>) currents. The I<sub>phasic</sub> occurs when a high concentration of GABA at the synapse binds to and activates postsynaptic GABA<sub>A</sub> receptors, resulting in IPSCs that are essential for spatially and temporally relevant inhibitory information transfer between neurons (Farrant and Nusser 2005; Mody 2005; Semeyanov et al. 2004). Extracellular GABA, diffusing away from the synaptic cleft after action potential (AP)–dependent or nonsynaptic GABA release, can also activate extrasynaptic or perisynaptic GABA<sub>A</sub> receptors. The continuous binding of extrasynaptic receptors by low concentrations of extracellular GABA generates a tonic inhibitory current (i.e., I<sub>tonic</sub>) in some neurons, which can potently regulate neuron function (Brickley et al. 1996; Keros and Hablitz 2005; Nusser and Mody 2002; Park et al. 2006; Semeyanov et al. 2003).

The amplitude of the I<sub>tonic</sub> is regulated by the expression characteristics of extrasynaptic GABA<sub>A</sub> receptors as well as by the ambient GABA concentration (Semeyanov et al. 2003), which is negatively regulated by a GABA transporter (GAT) system. There are three high-affinity GATs (GAT-1–3) expressed in the CNS (Borden 1996; Sarup et al. 2003). GAT-1 is the most widely expressed in neurons (Borden 1996; Minelli et al. 1995); GAT-2/3 are mainly expressed on glial cells, but can also be expressed on neurons under some circumstances (Borden 1996; Sarup et al. 2003). The activity of GATs can regulate neuronal excitability by modulating extracellular GABA concentration (Ortinski et al. 2006; Rossi et al. 2003).

The dorsal motor nucleus of the vagus (DMV) of the dorsal caudal brain stem is composed mainly of parasympathetic preganglionic motor neurons comprising vagal projections to most subdiaphragmatic viscera. It is located adjacent to the nucleus tractus solitarius (NTS), which relays visceral sensory information from the gastrointestinal (GI) and other systems to the DMV, principally in the form of excitatory glutamatergic and inhibitory GABAergic synaptic connections (Davis et al. 2003; Derbenev et al. 2004; Travagli et al. 1991, 2006). In particular, the GABAergic regulation of DMV output potently modulates visceral function, especially that of the GI system (Travagli et al. 2006). Modulation of GABA receptors in the DMV has significant effects on preganglionic motor neuron activity and consequently on the GI tract, but the potential contribution of I<sub>tonic</sub> to DMV neuron function has not been investigated. This study aimed to identify and characterize the tonic GABA current in DMV neurons, its modulation by GATs, gabazine, and gaboxadol (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol [THIP]), and its effects on membrane potential and AP firing activity in DMV neurons.

METHODS

Animals

Male Sprague–Dawley rats (Harlan, Indianapolis, IN), 4–8 wk of age, were housed under a standard 12-h light/dark cycle, with food and water provided without restriction. All animals were treated and cared for in accordance with National Institutes of Health guidelines...
and were approved by the Institutional Animal Care and Use Committees of Tulane University and the University of Kentucky.

**Stomach inoculation with PRV-152**

For some experiments, a fluorescently labeled viral vector, which selectively labels neurons in a transynaptic, retrograde manner, was used to identify gastric-related neurons for patch-clamp recordings and neuron labeling in slices (Glatzer et al. 2003; Smith et al. 2000). Under sodium pentobarbital anesthesia (Nembutal, 50 mg/kg; administered intraperitoneally; Abbott Laboratories, North Chicago, IL.), a laparotomy was performed and the gastric musculature was injected with an attenuated (Bartha) strain of pseudorabies virus (PRV-152, generously supplied by Dr. L. W. Enquist), which was constructed to express enhanced green fluorescent protein (EGFP). As previously described (Card et al. 1990; Glatzer et al. 2003), three to five 1-μl injections of PRV-152 (1–2 × 10^5 pfu/ml) were made into the gastric wall on the ventral surface of the corpus by using a 10-μl Hamilton syringe fitted with a 26-gauge needle. Each injection was made over a 1-min period and the needle was left in place for an additional 30 s at each site before removal. Animals were maintained in a biosafety level 2 laboratory for ≈91 h postinoculation, where they were allowed to recover. Food and water consumption was monitored to verify normal feeding behavior. This procedure allowed for labeling of a relatively large population of cells in the DMV that were specifically related to gastric motor function.

**Brain stem slice preparation**

Whole cell patch-clamp recordings were made using brain stem slices prepared from male Sprague–Dawley rats, 4–8 wk of age. Animals were deeply anesthetized by halothane (Sigma, St. Louis, MO) or isoflurane (Minrad, Buffalo, NY) inhalation to effect and then decapitated. The brain was removed and blocked on an ice-cold stand and the medulla and cerebellum were glued to a sectioning stage. Technical Products, St. Louis, MO). The ACSF contained (in mM): 124 NaCl, 3 KCl, 2 CaCl2, 1.3 MgCl2, 1.4 NaH2PO4, 26 NaHCO3, 11 glucose, and 1 kynurenic acid (pH 7.2–7.4, with an osmolality of 290–315 mOsm/kg). Slices were then incubated for ≥1 h in warm (32–35°C) oxygenated ACSF. For recording, a single brain slice was transferred to a chamber mounted on a fixed stage under an upright microscope (Model BX51WI; Olympus, Melville, NY), where they were continually superfused by warmed (30–33°C) oxygenated ACSF.

**Electrophysiological recording**

Whole cell voltage-clamp recordings were obtained in the DMV using patch pipettes with open tip resistances of 3–5 MΩ. The recording pipette was filled with a 130–140 Cs-gluconate (or K-gluconate), 10 HEPES, 2–4 Mg-ATP, and 0.1% biocytin. Cs+ was used as the primary cation carrier in voltage-clamp recordings, which blocked K+ currents, including GABA_B receptor-mediated currents. Neurons in the DMV were targeted for recording under a 40× water-immersion objective (numerical aperture = 0.8) with fluorescence and infrared-differential interference contrast (IR-DIC) optics, as previously described (Davis et al. 2003; Derbeney et al. 2004). For recordings from EGFP-labeled DMV neurons (i.e., neurons that were infected retrogradely from the stomach with PRV-152), initial visualization was made briefly under epifluorescence by using a fluorescein isothiocyanate filter set. The epifluorescence illumination was then stopped and IR-DIC illumination was used to guide the recording pipette onto the cell for whole cell recording, exactly as for recordings in unlabeled neurons. Recorded neurons were visualized and their EGFP content was documented on-line using a charged-coupled device camera and/or post hoc verification of EGFP in biocytin-filled neurons, as described previously (Davis et al. 2003; Smith et al. 2000).

Electrophysiological signals were obtained by using an Axopatch 200B amplifier or 700B amplifier (Molecular Devices, Union City, CA), digitized at 88 kHz (Neuroquest; Cygnus Technology, Delaware Water Gap, PA), low-pass filtered at 2 kHz, and recorded onto videotape and to a computer (Digidata 1320A, Molecular Devices) using pClamp 10.1 software (Molecular Devices). Recordings were analyzed with pClamp programs (Molecular Devices) or MiniAnalysis (Synaptosoft, Decatur, GA). Seal resistance was typically 2–5 MΩ and monitored periodically during the recording. Recordings were discarded if series resistance changed by >20% over the course of the experiment. Input resistance was assessed by measuring the voltage response to current steps (−40 to +5 pA; 400–800 ms) applied through the recording electrode in current-clamp recordings (K-glucuronate intracellular solution) or was estimated by measuring the current at the end of brief (20–400 ms) voltage pulses of 5–10 mV in voltage-clamp mode. Resting membrane potential was determined by monitoring the voltage at which no current was injected (i.e., removing voltage-clamp control of the neuron by switching to I = 0) during the recording.

**Data analysis**

IPSCs or miniature (m)IPSCs were analyzed with MiniAnalysis (Synaptosoft) to measure the peak amplitude, frequency, and decay time constant. Curve fitting for IPSC decay time was performed using the biexponential equation from MiniAnalysis, \( y = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \). In this equation, \( y \) is the peak amplitude, \( A_1 \) and \( A_2 \) are the amplitudes of the fast and slow decay components, and \( \tau_1 \) and \( \tau_2 \) are the corresponding decay time constants of \( A_1 \) and \( A_2 \) (Yeung et al. 2003). We used a weighted time constant to compare decay times between different experimental conditions. The weighted time constant of current decay times were calculated with the equation \( \tau_{\text{w}} = \sum A_i \tau_i \), Banks and Pearce 2000; Yeung et al. 2003). The resting \( I_{\text{leak}} \) amplitude was calculated as the difference between the steady-state holding current before and after application of 30 μM bicuculline or 100 μM picrotoxin. The total drug-induced \( I_{\text{leak}} \) amplitude describes the current blocked by GABA_A antagonists applied in the presence of agonists and/or GAT blockers. The holding current was calculated averaged using the mean of 30 segments (100 ms/segment; 1 segment/s) that were free of spontaneous (s)IPSCs (Brickley et al. 1996; Keros and Hablitz 2005; Nusser and Mody 2002). The mean phasic current (integrated current of IPSCs) was measured using the equation: \( I_{\text{phasic}} = f \times Q \), where \( f \) is the synaptic frequency and \( Q \) is the charge transfer measured as the area under the sIPSC (Nusser and Mody 2002; Park et al. 2006; Semyanov et al. 2003). Pooled results of single comparisons (e.g., before and after a single drug treatment) were analyzed using a two-tailed t-test; comparisons using multiple groups of cells or multiple tests within cells were compared using one-way ANOVA followed by Tukey’s post hoc test. Statistical significance for all measures was set at \( P < 0.05; \) F-scores are included in the text when ANOVA was used. Statistical measurements were performed with Microsoft Excel (Microsoft, Redmond, WA) or Prism (GraphPad Software, La Jolla, CA). Numbers were expressed as mean ± SE. All drugs were obtained from Sigma, Tocris Bioscience (Ellisville, MO), or Fisher Biosciences (Pittsburgh, PA). Tetrodotoxin (TTX, 1 μM), gabazine (0.5–25 μM), bicuculline methiodide (30 μM), picrotoxin (100 μM), strychnine (10 μM), GABA (5 μM), nipeptic acid (1 mM), SNAP-5114 (50 μM), NO-711 (10 μM), SKF89976A (30 or 90 μM), B-alanine (100 or 200 μM), and THIP (0.1–10 μM) were added to ACSF for specific experiments.
RESULTS

Identification of $I_{\text{tonic}}$ and relationship to $I_{\text{phasic}}$

In control ACSF, adding bicuculline (30 μM) resulted in a net inward current (Fig. 1A), revealing the total resting $I_{\text{tonic}}$ mediated by GABA$_A$ receptors. To determine the relationship between inhibitory synaptic currents and extrasynaptic $I_{\text{tonic}}$, we compared the amplitudes of the $I_{\text{tonic}}$ and the mean $I_{\text{phasic}}$ $(f \times Q)$ in 38 DMV neurons. In these neurons, the mean $I_{\text{tonic}}$ amplitude was 18.8 ± 2.1 pA (range: 0–46.5 pA; $n = 38$). The charge transfer (i.e., measured as area under the curve) for averaged sIPSCs was 0.4 ± 0.02 pC ($n = 38$). With an average frequency of $2.7 \pm 0.4$ Hz, this indicated a mean $I_{\text{phasic}}$ amplitude of 1.1 ± 0.2 pA (range: 0.1–5.5 pA; $n = 38$). A plot of the mean $I_{\text{phasic}}$ versus $I_{\text{tonic}}$ amplitude from each cell revealed a lack of correlation ($r^2 = 0.02$; Fig. 1B), suggesting that the $I_{\text{tonic}}$ amplitude did not arise from the summation of phasic currents or depend directly on the degree of synaptic inhibition. Further, this result suggested that the $I_{\text{tonic}}$ and $I_{\text{phasic}}$ were mediated by different receptor pools with distinct functional properties.

TTX decreased the $I_{\text{tonic}}$

We tested whether the extracellular GABA arose from AP-dependent GABA release in DMV neurons. Perfusion of TTX (1 μM) significantly decreased the amplitude of $I_{\text{tonic}}$ ($n = 8$) compared with $I_{\text{tonic}}$ in the absence of TTX ($P < 0.05$; Fig. 1, C and D). In the presence of TTX, the $I_{\text{tonic}}$ in 4/8 DMV neurons was very close to 0 pA (i.e., <4 pA) and the $I_{\text{tonic}}$ in the remaining neurons ranged from 4 to 12 pA. The average $I_{\text{tonic}}$ in TTX was 5.3 ± 2.0 pA. Nippecotic acid (1 mM) significantly elevated the $I_{\text{tonic}}$ to 17.5 ± 3.8 pA ($P < 0.05; n = 8$; Fig. 1, C and D), but this was significantly less than the $I_{\text{tonic}}$ in nipeotic acid under control conditions without TTX (44.4 ± 5.0 pA; $P < 0.05$). These results suggest that much of the extracellular GABA in DMV comes from AP-dependent synaptic release.

Effects of GABA transporter antagonists

Nipeotic acid (1 mM), a nonselective GAT blocker that would be expected to increase extracellular GABA concentration and may also act as a direct GABA$_A$ receptor agonist (Barrett-Jolley 2001; Keros and Hablitz 2005; Park et al. 2006), induced an outward current in 9/10 DMV neurons (20.1 ± 3.7 pA). Adding bicuculline (30 μM) revealed a total $I_{\text{tonic}}$ that was greater than the nipeotic acid–induced current (44.4 ± 5.0 pA). Overall, the $I_{\text{tonic}}$ revealed by bicuculline was increased from a resting value of 24.2 ± 3.1 to 44.4 ± 5.0 pA in nipeotic acid ($P < 0.05$; $n = 10$; Fig. 2, A and B).

Blocking GABA transport by addition of the GAT-2/3 blocker SNAP-5114 (50 μM), the GAT-1 blocker NO-711 (10 μM), or a combination of the two drugs did not significantly increase the resting $I_{\text{tonic}}$ in DMV neurons. Neither SNAP-5114 (2.3 ± 1.3 pA; range: −0.1 to 7.4 pA; $n = 6$; Fig. 2D), NO-711 (−0.2 ± 2.0 pA; $n = 5$), nor the combination of SNAP-5114 and NO-711 (1.1 ± 1.4 pA; $n = 6$) induced a significant degree of $I_{\text{tonic}}$. The effects on $I_{\text{tonic}}$ of these antagonists alone or in combination were significantly less than that observed in nipeotic acid ($F_{6,37} = 7.94; P < 0.05$; Fig. 2D).

SKF89976A is a derivative of nipeotic acid and also blocks GAT-1, whereas β-alanine, an endogenous amino acid, is a substrate for GAT-2/3 (Borden 1996; Dalby 2003). The combination of SKF89976A (30 or 90 μM) and β-alanine (100 or 200 μM) resulted in a 5.4 ± 2.5 pA increase in $I_{\text{tonic}}$ ($n = 5$; Fig. 2D), which was also significantly less than the effect of nipeotic acid ($P < 0.05$).

Nipeotic acid is a substrate for GATs and it can be imported into cells and exchanged with GABA, causing intracellularly stored GABA to move to the extracellular space over time (Dalby 2003; Keros and Hablitz 2005; Szerb 1982). Extracellular GABA levels can regulate the activity of GATs, indicating that the activity of GATs depends on the extracellular GABA concentration (Bernstein and Quick 1999; Ortinski et al. 2006). We therefore reasoned that GAT activity required a threshold GABA

FIG. 1. Relationship between type A γ-aminobutyric acid (GABA$_A$) receptor-mediated tonic ($I_{\text{tonic}}$) currents, synaptic currents, and action potential (AP)–dependent GABA release. A: the $I_{\text{tonic}}$ under baseline conditions was revealed by addition of bicuculline (30 μM). B: plot showing the relationship between $I_{\text{tonic}}$ amplitude and the mean $I_{\text{phasic}}$ in 38 DMV (dorsal motor nucleus of vagus) neurons. There was little correlation ($r^2 = 0.02$), indicating that different receptor pools mediate $I_{\text{tonic}}$ and $I_{\text{phasic}}$ synaptic currents. C: nipeotic acid (1 mM) increased the $I_{\text{tonic}}$ only slightly in the presence of tetrodotoxin (TTX, 1 μM); adding bicuculline (30 μM) revealed the total $I_{\text{tonic}}$. D: graph indicating that TTX produced a significant suppression of the $I_{\text{tonic}}$ compared with control. Nipeotic acid enhanced the $I_{\text{tonic}}$ in the presence of TTX, but the $I_{\text{tonic}}$ was significantly smaller in amplitude than that in the absence of TTX. Asterisks indicate significant difference between groups indicated (unpaired t-test; $P < 0.05$); double asterisks indicate significant effect of TTX within cells (paired t-test; $P < 0.05$). Number of replicates is in parentheses above each bar.
concentration to be effective. Adding GABA alone (5 μM) increased the $I_{\text{tonic}}$ by 7.8 ± 3.1 pA (range: 1.3–19.6 pA; $n = 6$; Fig. 2D). In the presence of GABA (5 μM), addition of NO-711 (10 μM) and SNAP-5114 (50 μM) together caused a significant increase in the $I_{\text{tonic}}$ in 7/8 DMV neurons (Fig. 2E). The $I_{\text{tonic}}$ in these 7 DMV neurons was increased from a resting value of 23.9 ± 4.16 pA to 95.0 ± 22.3 pA in the additional presence of GABA (5 μM) (Fig. 2F). The mean net increase in $I_{\text{tonic}}$ amplitude was 71.1 ± 19.4 pA (range: 6.4–160.8 pA; $n = 7$). This result suggested that GAT-1 and/or GAT-2/3 are expressed in DMV and play an important role in GABA reuptake, but their activity requires elevated ambient GABA concentration. In the presence of GABA (5 μM), addition of NO-711 (10 μM) alone increased the $I_{\text{tonic}}$ by only 4.2 ± 2.1 pA (range: −0.8–14.1 pA; $n = 6$). Addition of SNAP-5114 alone (50 μM) also failed to increase $I_{\text{tonic}}$ amplitude in 4/5 DMV neurons (−0.5 ± 1.2 pA; $n = 4$). The remaining neuron showed a 41.5 pA elevation in $I_{\text{tonic}}$. The $I_{\text{tonic}}$ induced by NO-711 plus SNAP-5114 together was significantly greater than that caused by either NO-711 or SNAP-5114 alone ($P < 0.05$; Fig. 2G). These results indicate that both GAT-1 and GAT-2/3 are expressed in DMV and they cooperate to regulate GABA concentration.

The analysis of sIPSCs before and after GAT blockers (with or without 5 μM GABA) revealed that the amplitude, frequency, and the weighted decay time of sIPSCs were not significantly changed by these agents ($P > 0.05$; see Table 1). The average amplitude of sIPSCs was 26.4 ± 1.2 pA and the frequency was 2.2 ± 0.2 Hz in 40 DMV neurons. The decay time was fitted well with a single-exponential in 29/40 DMV neurons. The decay time was 0.2 ms in these recordings. See Table 1 for complete comparison of effects of GAT blockers on IPSCs.

Sensitivity of $I_{\text{tonic}}$ to GABA$_A$ receptor antagonists

Gabazine, a competitive GABA$_A$ receptor antagonist, has been used to differentiate receptor pools mediating $I_{\text{tonic}}$ from $I_{\text{phasic}}$ in a number of studies (Bai et al. 2001; Park et al. 2006; Semyanov et al. 2003; Yeung et al. 2003). GABA currents

FIG. 2. The effects of GABA transporter blockers on $I_{\text{tonic}}$ in DMV neurons. A: application of nipeocotic acid (1 mM), a nonelective GABA transporter inhibitor, significantly increased the amplitude of $I_{\text{tonic}}$. B: graph showing the $I_{\text{tonic}}$ amplitude, revealed by adding bicuculline (30 μM), under control conditions and in the presence of nipeocotic acid. Asterisk indicates significant difference from control ($P < 0.05$; paired t-test). Number of replicates is in parentheses above each bar. C: in the presence of GABA (5 μM), addition of GABA (5 μM) also failed to increase the $I_{\text{tonic}}$ amplitude. Adding GABA alone (5 μM) increased the $I_{\text{tonic}}$ from a resting value of 23.9 ± 4.16 pA to 95.0 ± 22.3 pA in the additional presence of GABA (5 μM), under control conditions and in the presence of nipeocotic acid. Asterisk indicates significant difference from control ($P < 0.05$; paired t-test). Number of replicates is in parentheses above each bar. D: comparison of the $I_{\text{tonic}}$ amplitude changes induced by nipeocotic acid (1 mM), GABA (5 μM), NO-711 (10 μM), SNAP-5114 (50 μM), NO-711 + SNAP-5114, and the combination of SKF-9976A (30 or 90 M) with $\beta$-alanine (100–200 μM); strychnine 10 μM was preapplied into the artificial cerebrospinal fluid [ACSF] to block glycine receptors. Asterisk indicates significant difference vs. $I_{\text{tonic}}$ change in other groups [ANOVA; $F_{(5,37)} = 7.94$; $P < 0.05$]. Number of replicates is in parentheses above each bar. E: in the presence of GABA (5 μM), coapplication of NO-711 (10 μM) and SNAP-5114 (50 μM) increased the $I_{\text{tonic}}$. Adding bicuculline (30 μM) revealed the total $I_{\text{tonic}}$. F: graph summarizing the $I_{\text{tonic}}$ in 5 μM GABA and the total $I_{\text{tonic}}$ in the presence of NO-711 + SNAP-5114 and GABA. Asterisk indicates significant difference from GABA alone ($P < 0.05$). G: comparison of the $I_{\text{tonic}}$ amplitude changes induced by NO-711 + SNAP-5114, NO-711 (10 μM) alone, and SNAP-5114 (50 μM) alone in the presence of GABA (5 μM). Asterisk indicates significant difference vs. other groups ($P < 0.05$). Number of replicates is in parentheses above each bar.
Effects of GABA transporter blockers and THIP on sIPSCs

<table>
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<tr>
<th>Treatment (n)</th>
<th>Amplitude, pA</th>
<th>Frequency, Hz</th>
<th>Decay Time, ms</th>
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<tr>
<td></td>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
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<tr>
<td>NO-711 (5)</td>
<td>25.7 ± 3.9</td>
<td>24.2 ± 3.0</td>
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<td>SNAP-5114 (8)</td>
<td>32.5 ± 3.3</td>
<td>29.5 ± 1.5</td>
<td>1.8 ± 0.5</td>
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<td>NO-117 + SNAP-5114 (6)</td>
<td>27.0 ± 2.2</td>
<td>26.6 ± 2.3</td>
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<tr>
<td>SKF-89976A + β-alanine (6)</td>
<td>21.1 ± 3.0</td>
<td>19.8 ± 2.3</td>
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<td>GABA (6)</td>
<td>19.5 ± 3.7</td>
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<td>Nippecotic acid (7)</td>
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<td>NO-711 in GABA (5)</td>
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<td>25.1 ± 3.5</td>
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<tr>
<td>SNAP-5114 in GABA (6)</td>
<td>24.7 ± 0.7</td>
<td>25.1 ± 1.2</td>
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<td>NO-711 + SNAP-5114 in GABA (8)</td>
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<td>25.3 ± 2.2</td>
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<tr>
<td>THIP (8)</td>
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<td>26.0 ± 1.9</td>
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<td>THIP in TTX (5)</td>
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Values are means ± SE. Decay time is weighted decay time. *Denotes significant difference versus control value (P < 0.05); number of replicates in parentheses.

were measured in the presence of 2 or 25 μM gabazine. Gabazine (2–25 μM) blocked sIPSCs without changing the resting $I_{\text{tonic}}$ ($P > 0.05$; $n = 5$; Fig. 3A), which was revealed by adding bicuculline (30 μM). The amplitude of resting $I_{\text{tonic}}$ was 29.4 ± 6.9 pA. The amplitude of current that remained in the presence of gabazine was 28.5 ± 6.9 pA ($P > 0.05$; Fig. 3B). This result suggested that the receptors mediating $I_{\text{tonic}}$ and $I_{\text{phasic}}$ are not the same type in this set of neurons.

In another set of cells, the GABA transporter blocker nipeptic acid (1 mM) was added to increase extracellular GABA concentration (see Fig. 2A). The $I_{\text{tonic}}$ amplitude was increased to 40.9 ± 6.4 pA in the presence of nipeptic acid and addition of low concentrations of gabazine (0.5 or 1 μM) decreased it to 22.7 ± 5.6 pA [$F_{(3,33)} = 3.64; P < 0.05; n = 10$; Fig. 3, C and D], corresponding to a 46.3 ± 8.8% decrease. The occurrence of sIPSCs was effectively blocked in low concentrations of gabazine (0.5 μM), being decreased from 2.1 ± 0.5 Hz to 0.2 ± 0.1 Hz in gabazine ($P < 0.05$; $n = 10$; Fig. 3E). The amplitude of the gabazine-resistant $I_{\text{tonic}}$ component (22.7 ± 5.6 pA) was similar to the amplitude of the resting $I_{\text{tonic}}$ prior to being enhanced by nipeptic acid (28.5 ± 6.9 pA in gabazine; $P = 0.47$). This suggested that the...
high-affinity, gabazine-insensitive GABA<sub>A</sub> receptors mediating the I<sub>tonic</sub> under basal conditions are nearly completely saturated. Increasing GABA concentration with nipecotic acid revealed a gabazine-sensitive I<sub>tonic</sub> in addition to the resting I<sub>tonic</sub> that was unaffected by gabazine.

We next tested whether a higher concentration of gabazine (i.e., 25 μM) would block I<sub>tonic</sub> in the presence of nipecotic acid (1 mM). There was no difference between the amplitude of I<sub>tonic</sub> measured in 0.5 μM gabazine (22.5 ± 7.5 pA) versus that measured in 25 μM gabazine [20.6 ± 6.7 pA; n = 7; F<sub>(2,33)</sub> = 3.64; P > 0.05; Fig. 3D]. These data indicated that when the I<sub>tonic</sub> in DMV was increased by blocking GABA transport and effectively increasing extracellular GABA concentration, the tonic component mediated by lower-affinity, gabazine-sensitive GABA<sub>A</sub> receptors could be completely blocked by low concentrations of gabazine (0.5–1 μM). However, a bicuculline-sensitive I<sub>tonic</sub> component remained evident even when gabazine concentration was increased.

**Effects of THIP on I<sub>tonic</sub>**

THIP is a muscimol derivative and a hypnotic drug used in many studies to help differentiate synaptic from extrasynaptic GABAA receptors (Chandra et al. 2006; Drasbek et al. 2007; Jia et al. 2005). In our experiments, THIP increased the I<sub>tonic</sub> in a concentration-dependent manner (Fig. 4E). At low concentration, THIP (0.1 μM) had little effect on the I<sub>tonic</sub> amplitude (0.4 ± 2.0 pA; n = 4); THIP (1 μM) increased the I<sub>tonic</sub> by 8.4 ± 1.4 pA (n = 4) and, at 10 μM, THIP further increased the I<sub>tonic</sub> in 8/8 DMV neurons (95.1 ± 23.3 pA; Fig. 4B) from a resting value of 18.0 ± 5.3 to 113.1 ± 24.4 pA (P < 0.05; n = 8; Fig. 4D).

The frequency of sIPSCs was significantly reduced by THIP (10 μM) from 2.1 ± 0.4 to 0.9 ± 0.1 Hz (n = 8; P < 0.05; Table 1). Addition of TTX prevented the effects of THIP on the frequency of sIPSCs (n = 5; P > 0.05), suggesting the effect was on activity of afferent intact GABA neurons. The amplitude and τ<sub>rec</sub> of sIPSCs were not significantly altered by THIP, either alone or in TTX (P > 0.05), indicating that THIP did not potentiate postsynaptic GABA<sub>A</sub> receptors mediating I<sub>phasic</sub>.

Since a large proportion of DMV cells participate in regulating the GI tract and modulation of GABA<sub>A</sub> receptor activity in the DMV can affect gastric function, we determined the effects of THIP on I<sub>tonic</sub> in retrogradely labeled, GI-related DMV neurons after PRV-152 inoculation of the stomach wall. Labeled neurons were targeted for whole cell patch-clamp recording in slices 72–91 h after inoculation (Fig. 4A). THIP (10 μM) significantly increased the I<sub>tonic</sub> in 8/8 gastric-related DMV neurons. The I<sub>tonic</sub> was increased from a resting level of 13.9 ± 3.6 to 76.9 ± 10.6 pA by THIP (10 μM; P < 0.05; n = 8; Fig. 4, C and D). No significant difference was observed for the resting (P = 0.5) or THIP-induced I<sub>tonic</sub> in THIP (P = 0.2) in PRV-152–labeled neurons versus unlabeled DMV neurons, but this result indicated that gastric-related neurons express a THIP-modulated I<sub>tonic</sub>.

**Nipecotic acid and THIP changed AP activity in DMV neurons**

In several brain areas, I<sub>tonic</sub> plays an important role in regulating neuron excitability (Brickley et al. 1996; Park et al. 2006; Semyanov et al. 2003). In current-clamp recordings, perfusion of nipecotic acid (1 mM) hyperpolarized the resting
membrane potential \([F_{(2,20)} = 7.31; P < 0.05]\) and/or reduced the AP firing activity \([F_{(2,32)} = 4.08; P < 0.05; \text{Fig. 5, D and E}]\). This occurred by increasing \(I_{\text{tonic}}\) but not phasic currents on DMV neurons because phasic currents (sIPSCs) were not altered by nipecotic acid (Table 1). Three of 13 DMV neurons did not fire APs at resting membrane potential and they were not analyzed for frequency changes. The AP frequency in 10 spontaneously firing DMV neurons was \(1.3 \pm 0.5 \text{ Hz} (n = 7; \text{Fig. 5E})\). Resting membrane potential was unchanged in the remaining 6 neurons; the overall effect of nipecotic acid on membrane potential of responding and nonresponding cells combined was not significant \((n = 13; P > 0.05)\). Addition of picrotoxin (100 \(\mu M; n = 4\)) or bicuculline (30 \(\mu M; n = 6\)) reversed the inhibitory effects caused by nipecotic acid, increasing AP frequency to \(1.8 \pm 0.5 \text{ Hz} (n = 10)\).

The resting membrane potential was hyperpolarized by nipecotic acid (1 mM) in 7 of 13 DMV neurons. The average resting membrane potential \((-51.8 \pm 2.1 \text{ mV})\) was hyperpolarized to \(-55.4 \pm 2.3 \text{ mV}\) in nipecotic acid \((P < 0.05; n = 7; \text{Fig. 5D})\). Resting membrane potential was unchanged in the remaining 6 neurons; the overall effect of nipecotic acid on membrane potential of responding and nonresponding cells combined was not significant \((n = 13; P > 0.05)\). Addition of picrotoxin \((100 \mu M; n = 4\)) or bicuculline \((30 \mu M; n = 9\)) depolarized the resting membrane potential to \(-47.3 \pm 1.9 \text{ mV} (P < 0.05; n = 13; \text{Fig. 5D})\). Nipecotic acid (1 mM) also decreased the input resistance by \(>10\% \) in 7/14 DMV neurons in whole cell current-clamp recordings from 1,112 \pm 94 to 906 \pm 69 \(\Omega\) \([F_{(2,20)} = 6.15; P < 0.05; n = 7; \text{Fig. 6, A and B}]\). Input resistance partially recovered subsequent to bicuculline (30 \(\mu M\)) or picrotoxin (100 \(\mu M\)) application \((982 \pm 117 \Omega; n = 7; \text{Fig. 6B})\). Nipecotic acid changed input resistance by \(<10\% \) in the remaining 7 cells; the overall effect of nipecotic acid on input resistance of responding and nonresponding cells combined was also significant \((n = 14; P < 0.05)\), as was the effect of blocking GABA_A receptors \((n = 14; P < 0.05)\). The effect of THIP (10 \(\mu M\)) on AP firing was determined. Resting membrane potential \((-53.0 \pm 1.2 \text{ mV})\) was hyperpolarized to \(-55.8 \pm 1.1 \text{ mV}\) by THIP \([F_{(2,38)} = 25.36; P < 0.05; n = 13; \text{Fig. 5, A and B}]\). Addition of bicuculline depolarized the resting membrane potential to \(-48.8 \pm 1.8 \text{ mV} (P < 0.05; n = 13)\). THIP abolished the AP firing in 10/13 DMV neurons and decreased activity in the remaining 3 DMV neurons. The average firing frequency at resting membrane potential was \(1.77 \pm 0.29 \text{ Hz}\) and was decreased to \(0.08 \pm 0.06 \text{ Hz}\) in THIP \([F_{(2,38)} = 41.24; P < 0.05; n = 13; \text{Fig. 5C}]\). Addition of bicuculline (30 \(\mu M\)) increased AP frequency to \(1.95 \pm 0.25 \text{ Hz} (P < 0.05; n = 13)\). THIP also significantly decreased the whole cell input resistance from \(802 \pm 545 \Omega\) \([F_{(2,17)} = 41.18; P < 0.05; n = 6; \text{Fig. 6C}]\).

**FIG. 5.** Effects of THIP and nipecotic acid on AP firing. A: current-clamp recording at resting membrane potential showing that THIP (10 \(\mu M\)) suppressed APs and hyperpolarized the membrane potential; bicuculline (30 \(\mu M\)) reversed the effect of THIP. B and C: bar graphs summarizing the effects of THIP and bicuculline on the resting membrane potential \((\text{B})\) and AP frequency \((\text{C})\). D and E: summary graphs indicating that nipecotic acid (1 mM) also suppressed APs and hyperpolarized the resting membrane potential; picrotoxin (100 \(\mu M\)) or bicuculline (30 \(\mu M\)) blocked the effects of nipecotic acid. Asterisks indicate significant differences between groups indicated \((\text{ANOVA}; P < 0.05)\). Replicate number in parentheses.
After adding bicuculline, the whole cell input resistance recovered to 837 ± 77 MΩ (P < 0.05; n = 6).

**DISCUSSION**

These results describe a tonic GABA<sub>AA</sub> receptor-mediated inhibitory current expressed in DMV neurons. Although the $I_{\text{tonic}}$ required synaptic release, it did not depend on the degree of $I_{\text{phasic}}$, indicating that it was not mediated by summation of IPSCs. However, it was reduced by addition of TTX, indicating the ambient GABA originated from AP-dependent release of neurotransmitter. Modulating $I_{\text{tonic}}$ with THIP or GAT blockers increased the $I_{\text{tonic}}$ in the DMV without altering IPSC amplitude, similar to descriptions in other brain areas (Jensen et al. 2003; Keros and Hablitz 2005; Nusser and Mody 2002; Rossi and Hamann 1998; Wei et al. 2003). The THIP-induced decrease in IPSC frequency seems likely to have been due to upstream inhibitory effects of THIP on afferent GABA neurons. Since these agents differentially affected phasic and tonic components of the GABA current, this also supports the hypothesis that $I_{\text{tonic}}$ involves activation of a separate set of receptors and is not simply due to summation of IPSCs. Transporter regulation of $I_{\text{tonic}}$ depended on the activity of both GAT-1 and GAT-2/3 and their involvement was revealed when extracellular GABA concentration was elevated. The GABA<sub>AA</sub> receptors mediating the $I_{\text{tonic}}$ displayed different sensitivity to gabazine than those mediating $I_{\text{phasic}}$ (i.e., IPSCs). The $I_{\text{phasic}}$ was sensitive to low concentrations of gabazine, whereas the GABA<sub>AA</sub> receptors mediating the $I_{\text{tonic}}$ were composed of two types: one type was gabazine insensitive and was nearly saturated under basal conditions. The other was gabazine sensitive and was activated when GABA concentration was elevated. Spontaneously opened GABA<sub>AA</sub> receptors, independent of the presence of agonist, can also produce an $I_{\text{tonic}}$ that is insensitive to gabazine, a competitive antagonist, but can be blocked by bicuculline and picrotoxin (McCartney et al. 2007). Expression of this type of $I_{\text{tonic}}$ is probably not predominant in DMV because the $I_{\text{tonic}}$ was markedly suppressed in TTX, suggesting that the $I_{\text{tonic}}$ in DMV is gated by released GABA under baseline conditions and not mediated by the spontaneously open GABA<sub>AA</sub> receptors. We cannot rule out the possibility of other GABA sources, such as nonvesicular GABA release or reversed transporters in addition to AP release. However, the GABA accumulated from AP-dependent presynaptic release is responsible for the largest part of the total $I_{\text{tonic}}$. The $I_{\text{tonic}}$ plays a major role in determining the overall excitability of DMV neurons, which can be regulated by nipecotic acid and THIP, relatively independent of any direct effects on IPSC amplitude.

**Multiple receptor types mediate $I_{\text{tonic}}$**

The $I_{\text{tonic}}$ can be mediated by more than one type of GABA<sub>AA</sub> receptor, which can be differently influenced by the external GABA concentration (Fritschy and Brunig 2003; Scimemi et al. 2005; Semyanov et al. 2003). The receptor subunit composition determines the pharmacological and kinetic properties of the GABA receptor. Receptors containing α4, α5, or α6 together with γ2 or δ subunits are primarily high affinity and located outside the synaptic junction, whereas receptors with α1, α2, or α3 and γ2 subunits are mainly present in synapses (Farrant and Nusser 2005; Vicini and Ortinski 2004). In adult rodents, α1, α3, and γ2 (i.e., putative synaptic) and α5, δ (i.e., putative extrasynaptic) subunit protein or message are expressed in the vagal complex (Milligan et al. 2004; Pirker et al. 2000), including DMV (Broussard et al. 1996, 1997). It thus...
seems likely that synaptic GABA<sub>Δ</sub> receptors in the DMV are composed of α1/2/3, β, and γ2 subunits. The extrasynaptic GABA<sub>Δ</sub> receptors with lower affinity for GABA may be of similar composition, whereas the high-affinity extrasynaptic receptors may be composed of α4/5/6, β, and γ or δ/ε subunits.

A recently developed sleep-promoting hypnotic drug, THIP is a muscimol derivative and can easily pass the blood brain barrier, but cannot be taken up by GATs (Harrison 2007; Rossi et al. 2003; Wafford and Ebert 2006). Muscimol and THIP were reported to cause vomiting in some studies (Beaumont et al. 2008; Wafford and Ebert 2006), suggesting activity in the brain stem. Due to its pharmacological properties, THIP is used in many studies to differentiate GABA<sub>Δ</sub> receptors containing δ and/or α4 subunits (Chandra et al. 2006; Drasbek et al. 2007; Jia et al. 2005). Here, THIP increased the I<sub>tonic</sub>, hyperpolarized membrane potential and decreased input resistance, similar to effects in the thalamus and dentate gyrus (Chandra et al. 2006).

A low concentration of THIP (0.1–1 μM) was used to test the putative existence of the δ subunit in extrasynaptic receptors (Cope et al. 2005; Drasbek et al. 2007) and may also affect α4-containing GABA<sub>Δ</sub> receptors in thalamus and dentate gyrus (Chandra et al. 2006). At higher concentrations (i.e., >3 μM), THIP is a subunit-nonsensitive GABA<sub>Δ</sub> receptor agonist (Brown et al. 2002; Jia et al. 2005; Ortinski et al. 2006). In our studies, THIP increased the I<sub>tonic</sub> in DMV neurons in a concentration-dependent manner, but an intermediate concentration (1 μM) was required to initiate significant effects. We therefore cannot rule out the possible expression of α4 and/or δ subunits in some GABA<sub>Δ</sub> receptors, but this kind of GABA<sub>Δ</sub> receptor may account for a relatively small percentage in the pool of extrasynaptic GABA<sub>Δ</sub> receptors.

THIP is a direct GABA<sub>Δ</sub> receptor agonist and its activity is restricted to extrasynaptic domains (Drasbek et al. 2007; Harrison 2007). Besides high-affinity extrasynaptic GABA<sub>Δ</sub> receptors, THIP also activates low-affinity extrasynaptic GABA<sub>Δ</sub> receptors, which may be composed of α1/2/3, β, and γ2 subunits, but do not directly influence synaptic GABA<sub>Δ</sub> receptors. Our finding that THIP did not increase sIPSC amplitude is consistent with effects on extrasynaptic receptors. In the DMV, THIP decreased the frequency of sIPSCs in a TTX-sensitive manner, which suggested an effect on APs in afferent GABAergic neurons, most likely originating in NTS (Davis et al. 2004). Our results are consistent with the conclusion that THIP acts on the extrasynaptic GABA<sub>Δ</sub> receptors in the vagal complex.

In hippocampal interneurons and pyramidal cells, there were two components of I<sub>tonic</sub> observed when GABA uptake was blocked (Semyanov et al. 2003), similar to our observations in DMV cells. One component of I<sub>tonic</sub> was sensitive to low gabazine concentration (0.5 μM), whereas another component was gabazine resistant (Semyanov et al. 2003). Under baseline conditions, however, hippocampal interneurons have only one component of I<sub>tonic</sub>, which was not affected by a low concentration of gabazine but blocked by picrotoxin (Semyanov et al. 2003), similar to our results in DMV. When GABA transporters were blocked, however, the I<sub>tonic</sub> in most DMV neurons had two components. One component was sensitive to a low concentration of gabazine (0.5 or 1 μM); the other component was resistant to gabazine, even at 25 μM, but was bicuculline sensitive. The subunit composition of GABA<sub>Δ</sub> receptors mediating the gabazine-sensitive component of I<sub>tonic</sub> may be similar in this regard to synaptic receptors. However, as in hippocampus (Semyanov et al. 2003), they are apparently not the same receptors, since nipeptic acid did not alter IPSCs and the degree of synaptic inhibition was not correlated with I<sub>tonic</sub> amplitude. This set of extrasynaptic GABA<sub>Δ</sub> receptors is mainly unbound under baseline control conditions, but when external GABA concentration was increased, these receptors were activated, contributing to the background I<sub>tonic</sub> in DMV. The gabazine-insensitive component may be mediated by higher-affinity extrasynaptic GABA<sub>Δ</sub> receptors. The amplitude of this component of the I<sub>tonic</sub> was similar to the resting I<sub>tonic</sub> in DMV neurons, suggesting that GABA<sub>Δ</sub> receptors mediating this part of I<sub>tonic</sub> may be nearly saturated under baseline conditions. In the presence of nipeptic acid, gabazine at a higher concentration (25 μM) did not provide improved blockade of the I<sub>tonic</sub> than did that at low concentrations. In cardiac vagal neurons of the nucleus ambiguus, I<sub>tonic</sub> persists in the presence of 25 μM gabazine, is unaffected by GAT-1 blockade, and expresses a benzodiazepine-dependent recruitment of a second I<sub>tonic</sub> species (Bouairi et al. 2006). When GABA was elevated in the present study, DMV neurons displayed both gabazine-sensitive and -insensitive components, suggesting that I<sub>tonic</sub> with multiple components may significantly modulate multiple vagal motor functions and could also indicate sensitivity of vagal afferents to gabazine, which is related to the functional projection of the neuron (e.g., the gut).

**GABA transporter modulation**

GATs exert their function by reuptake of GABA into neurons and glia, limiting the spillover of GABA from synapses, terminating synaptic transmission, and maintaining a stable extracellular GABA concentration (Borden 1996; Dalby 2003). In electrophysiological studies, GAT-1 blockers were found to increase the I<sub>tonic</sub> in hippocampus, thalamus, and dentate gyrus (Chandra et al. 2006; Jensen et al. 2003; Nusser and Mody 2002; Semyanov et al. 2003), but not in the present study or in cardiac vagal neurons of nucleus ambiguus (Bouairi et al. 2006). In neocortex, GAT-1 and GAT-2/3 cooperate to transport GABA out of extracellular space (Keros and Hablitz 2005) and this also appears to occur in the DMV. The activities of GATs are temperature sensitive and can be modulated by the extracellular level of GABA (Bernstein and Quick 1999; Ortinski et al. 2006). In addition to being a potential GABA<sub>Δ</sub> receptor agonist (Barrett-Jolley 2001), nipeptic acid is a substrate of GATs. It can also be transported into the cell and exchange GABA from inside to outside (Dalby 2003; Keros and Hablitz 2005; Szerb 1982), effectively increasing GABA concentration. Any of these mechanisms could contribute to the effectiveness of nipeptic acid in inducing I<sub>tonic</sub>. Coapplication of NO-711 and SNAP-5114 in the presence of 5 μM GABA remarkably enhanced the I<sub>tonic</sub> implying that extracellular GABA concentration was required to be elevated for GAT blockade to have an effect in the DMV. This result also suggests that both GAT-1 and GAT-2/3 are expressed in DMV and that they cooperate to regulate I<sub>tonic</sub> under circumstances of elevated GABA release.
GABAergic $I_{\text{tonic}}$ in vagal motor neurons

$I_{\text{tonic}}$ modulation, neuronal excitability, and physiological implications

Agonists or antagonists acting on GABA\(_A\) receptors alter the excitability of NTs and DMV neurons and, consequently, profoundly influence autonomic functions. These effects have traditionally been considered and measured in the context of synaptic GABA\(_A\) receptor activity, but clearly the influence of $I_{\text{tonic}}$ in the DMV requires further attention in the context of effects on the GI tract and other visceral functions. Microinjections of bicuculline into the DMV excite vagally mediated gastric motor function in cats and rats, including increases in intragastric pressure, gastric motility, and gastric secretory volume (Sivarao et al. 1998). Factors that affect inhibitory connections from the NTS to the DMV can potentially alter gastric motility by modulating vagal parasympathetic efferent neurons (Ferreira Jr et al. 2001; Travagl et al. 2006). Phasic currents play a critical role in mediating signals transmitted from one cell to another in a temporally meaningful way. Postsynaptic GABA\(_A\) receptors mediating $I_{\text{phasic}}$ are activated rapidly and in large quantity and then quickly desensitize (Farrant and Nusser 2005; Semyanov et al. 2004). The $I_{\text{phasic}}$ in DMV likely mediates vagal reflex signals from NTS to DMV, which are then transmitted to viscera via temporally relevant inhibition of motor neuron activity.

The $I_{\text{tonic}}$ arises from the continuous activation of extrasynaptic GABA\(_A\) receptors. It is thus present in DMV neurons and in other areas as a background inhibitory current that contributes to membrane potential and regulates neuronal excitability (Brickley et al. 1996; Farrant and Nusser 2005; Semyanov et al. 2004). The total conductance of the $I_{\text{tonic}}$ is estimated to be four- to fivefold that of the total $I_{\text{phasic}}$ in hippocampus and hypothalamus (Park et al. 2006; Scimemi et al. 2005; Semyanov et al. 2003). In our studies, $I_{\text{tonic}}$ in DMV contributes roughly 94% of the total ($I_{\text{tonic}} + I_{\text{phasic}}$) GABA\(_A\) receptor-mediated current. We also found that increasing $I_{\text{tonic}}$ amplitude with nipepticot acid or THIP had little effect on IPSC amplitude, although hyperpolarized DMV neurons decreased their firing frequency and decreased input resistance, similar to effects seen in cardiac vagal neurons (Bouairi et al. 2006). The $I_{\text{tonic}}$ may thus play an important role in determining the excitability of DMV neurons by preventing excessive excitation, inhibiting transmission of other synaptic signals, or regulating the overall excitability of the network and therefore the vagal motor system. Gastric motor neurons are pacemakers, firing slow, regular APs (Browning et al. 1999; Travagl et al. 1991). The degree of $I_{\text{tonic}}$, for example, likely contributes to the “pace” of neuron firing, thus regulating autonomic gastric tone. Physiological effects on visceral functions of differentially altering $I_{\text{tonic}}$ and $I_{\text{phasic}}$ in the DMV have never been examined. It may be possible to affect changes in vagal reflexes versus vagal tone by selectively altering one or the other type of GABA current. In addition, GABA mimetics are used to treat a variety of disorders (such as sleep dysfunction, epilepsy, and anxiety, for example). Exploration of possible effects on $I_{\text{tonic}}$ in DMV neurons may improve understanding of autonomic side effects sometimes observed with these drugs.

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