Kv1.3 channels regulate synaptic transmission in the nucleus of solitary tract

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Ramirez-Navarro A, Glazebrook PA, Kane-Sutton M, Padro C, Kline DD, Kunze DL. Kv1.3 channels regulate synaptic transmission in the nucleus of solitary tract. J Neurophysiol 105: 2772–2780, 2011. First published March 23, 2011; doi:10.1152/jn.00494.2010.—The voltage-gated K+ channel Kv1.3 has been reported to regulate transmitter release in select central and peripheral neurons. In this study, we evaluated its role at the synapse between visceral sensory afferents and secondary neurons in the nucleus of the solitary tract (NTS). We identified mRNA and protein for Kv1.3 in rat nodose ganglia using RT-PCR and Western blot analysis. In immunohistochemical experiments, anti-Kv1.3 immunoreactivity was very strong in internal organelles in the soma of nodose neurons with a weaker distribution near the plasma membrane. Anti-Kv1.3 was also identified in the axonal branches that project centrally, including their presynaptic terminals in the medial and commissural NTS. In current-clamp experiments, margatoxin (MgTx), a high-affinity blocker of Kv1.3, produced an increase in action potential duration in C-type but not A- or Ah-type neurons. To evaluate the role of Kv1.3 at the presynaptic terminal, we examined the effect of MgTx on tract evoked monosynaptic excitatory postsynaptic currents (EPSCs) in brain slices of the NTS. MgTx increased the amplitude of evoked EPSCs in a subset of neurons, with the major increase occurring during the first stimuli in a 20-Hz train. These data, together with the results from somal recordings, support the hypothesis that Kv1.3 regulates the duration of the action potential in the presynaptic terminal of C fibers, limiting transmitter release to the postsynaptic cell.

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THE VOLTAGE-GATED K+ CHANNEL Kv1.3, best known for its role in immunological responses in lymphocytes, is also present in select populations of central and peripheral neurons. Kv1.3 has been reported to be targeted primarily to axons (Veh et al. 1995; Rivera et al. 2005) and to be involved in the regulation of transmitter release (Ohno-Shosaku 1996; Shoudai et al. 2007; Doczi et al. 2008). A recent study (Gazula et al. 2010) has identified Kv1.3 in presynaptic terminals in the calyx of Held. In nodose ganglia (NG) visceral sensory neurons, a variety of voltage-gated K+ channels contribute to excitability, including the members of the Kv1 family Kv1.1, Kv1.2, and Kv1.6 (Glazebrook et al. 2002), the KCNQ family (Wladyka et al. 2002), and Kv2.1 (Glazebrook et al. 2002). While we find that these currents collectively account for the majority (>80%) of delayed rectifying and slowly inactivating outward K+ currents in these neurons, a component of this K+ current remains unblocked in the presence of α-dendrotoxin (DTx) to block Kv1.1, Kv1.2, and Kv1.6, and MgTx to block KCNQ2, KCNQ3, and KCNQ5, and an intracellular Kv2.1 blocking antibody. In surveying the mRNA expression of the K+ channels in NG neurons, we detected the presence of another member of the Kv1 family, Kv1.3. In this study, we asked where Kv1.3 protein is expressed in NG neurons and their axons and whether it plays a role in excitability at central terminals. Specifically, we asked whether Kv1.3 might regulate the duration of the action potential and, thus, potentially alter transmitter release at a presynaptic terminal.

Visceral sensory neurons have been subdivided according to various criteria. Historically, the population was divided into three groups based on the conduction velocity of the axons, action potential duration, and the resistance of voltage-gated Na+ current to TTX (Belmonte and Gallego 1983; Bossu and Feltz 1984; Stansfeld and Wallis 1985). The first group of neurons (A-type neurons) has axons that conduct in the range of fast myelinated fibers and have narrow action potentials, and their Na+ currents are blocked by TTX. The second group (Ah-type neurons) displays broader action potentials than A-type neurons, exhibits a small hump on the falling phase of the action potential, and have axons that conduct in the slow myelinated fiber range (Stansfeld and Wallis 1985, Li and Schild 2007b). Whereas the Ah-type group has not been examined specifically in NG neurons for TTX sensitivity, a comparable group of Ah-type neurons in dorsal root ganglion expresses TTX-resistant Na+ current as well as TTX-sensitive current (Villiére and McLachlan 1996). These first two cell groups with myelinated fibers are estimated to make up only 10–25% of the total population based on anatomic and physiological studies (Evans and Murray 1954; Agostoni et al. 1957; Mei et al. 1980; Higashi and Nishi 1982; Yamasaki et al. 2004; Li and Schild 2007b). Finally, the third group (C-type neurons) has the longest duration action potentials, and their voltage-gated Na+ currents are poorly blocked by TTX (Stansfeld and Wallis 1985; Bossu and Feltz 1984; Schild and Kunze 1997). These neurons have axons conducting in the range of unmyelinated fibers. In the present study, we classified the response of nodose neurons to margatoxin (MgTx), a blocker of Kv1.3, based on the duration of the action potential and the presence or absence of TTX-resistant current.

METHODS

Animal protocols. Animal protocols were approved by Institutional Animal Care and Use Committees of Case Western Reserve University and the University of Missouri, and animals were handled according to their guidelines. With the exception of the postnatal day 1 animals included in the Western blot analysis, all animals were male.
Kv1.3 MODULATES THE SENSORY AFFERENT-NTS SYNAPSE

Western blot analysis. Rat pups were anesthetized in a saturated CO₂ chamber before the collection of the ganglia. NGs from 10 newborn (postnatal day 1) rats were pooled and frozen at −80°C. Adult rats were anesthetized with 5% isoflurane and decapitated. NGs were isolated, frozen in liquid nitrogen, and kept at −80°C. The experiment was repeated in two separate groups of animals. Frozen NGs were homogenized in RIPA buffer (1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 0.15 M NaCl, 50 mM Tris-HCl, and 2.5 mM EDTA) complemented with protease inhibitors (Complete, mini-EDTA-free tablets, Roche, Indianapolis, IN) and phosphatase inhibitors (set I and II, Calbiochem, La Jolla, CA). Samples were incubated on ice for 2 h and then centrifuged at 14,000 g for 15 min at 4°C. The protein concentration of the supernatant was measured by the BCA method (Pierce, Rockford, IL). Equal amounts of protein were separated on 4–20% Tris-glycine gel (Invitrogen) and transferred to polyvinylidene difluoride membranes. Western blot analyses were performed as previously described (Kline et al. 2007). Anti-Kv1.3 (1:500, rabbit polyclonal, Alomone) and anti-actin (1:2,000) primary antibodies were used for the immunoblot analysis.

Antibodies. Two commercial antibodies against Kv1.3 were used in these experiments. The polyclonal antibody (APC-002, lot AN-03, Alomone Labs, Jerusalem, Israel) was generated against a glutathione S-transferase (GST) fusion protein corresponding to residues 471–523 of human Kv1.3 protein recognizes a single band (~65 kDa) on a Western blot (Fig. 1). The antibody preabsorbed with the immunizing peptide was tested in NT5 sections and gave no signal. We also used a monoclonal antibody [75-009, lot no. 413–5RR-07, clone L23.27lg2a, NeuroMab, University of California (Davis, CA)/National Institutes of Health/NeuroMab Facility (Davis, CA)] generated against a synthetic peptide corresponding to rat sequence 845–506. This antibody recognizes a band of ~70 kDa and has been tested in Kv1.3 knockout mice for specificity (NeuroMab). In addition, we used a monoclonal antivesicular glutamate transporter antibody (clone N29/29, fusion protein amino acids 501–582, NeuroMab), a goat polyclonal anti-actin antibody (sc-1616; lot no. H0608, Santa Cruz Biotechnology, Santa Cruz, CA), and a mouse monoclonal anti-mycin basic protein (MBP) antibody (MAB 381, amino acids 119–131, Chemicon, Millipore, Billerica, MA).

PCR amplification of Kv1.3 α-subunit cDNA fragments. mRNA from the adult rat NG and brain was isolated using the microPoly (A+) Pure Kit (Ambion). Poly-(A+) mRNA was quantified by spectrophotometric absorbance at 260 nm and stored at −80°C until use. Primers used to amplify the cDNA fragment corresponding to a region of the rat Kv1.3 gene (Accession No. RATRGK5) by RT-PCR were as follows: sense 5′-AGAGCAGCTTTGGAGCTGCCAAA-CAAC-3′ (695–721 bp) and antisense 5′-CCTCTTCTACATC-CATATACTCCGAC-3′ (1,487–1,463 bp). RT-PCR was performed as previously described (Glazebrook et al. 2002). Channel-specific PCR products were identified by hybridization using a radiolabeled internal oligonucleotide specific for the Kv1.3 channel. The internal oligonucleotide was 5′-GACAATCTGCGTTATCGTTAG-3′ (nucleotides 1,209–1,235). Southern blots were performed as previously described (Glazebrook et al. 2002; Doan et al. 2004).

Electrophysiology. Adult rats were anesthetized with 5% isoflurane and decapitated. The ganglia were dissected and placed in cold nodose complete media, which consisted of DMEM-F-12, 5% FBS (HyClone, Logan, UT), and 0.1% penicillin-streptomycin (Invitrogen, Grand Island, NY). The ganglia were subsequently treated in a saturated CO₂ chamber before the collection of the ganglia. NGs from 10 newborn (postnatal day 1) rats were pooled and frozen at −80°C. Adult rats were anesthetized with 5% isoflurane and decapitated. NGs were isolated, frozen in liquid nitrogen, and kept at −80°C. The experiment was repeated in two separate groups of animals. Frozen NGs were homogenized in RIPA buffer (1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 0.15 M NaCl, 50 mM Tris-HCl, and 2.5 mM EDTA) complemented with protease inhibitors (Complete, mini-EDTA-free tablets, Roche, Indianapolis, IN) and phosphatase inhibitors (set I and II, Calbiochem, La Jolla, CA). Samples were incubated on ice for 2 h and then centrifuged at 14,000 g for 15 min at 4°C. The protein concentration of the supernatant was measured by the BCA method (Pierce, Rockford, IL). Equal amounts of protein were separated on 4–20% Tris-glycine gel (Invitrogen) and transferred to polyvinylidene difluoride membranes. Western blot analyses were performed as previously described (Kline et al. 2007). Anti-Kv1.3 (1:500, rabbit polyclonal, Alomone) and anti-actin (1:2,000) primary antibodies were used for the immunoblot analysis.
MgCl₂, 2.0 CaCl₂, 10.0 glucose, and 10 HEPES (pH 7.4) and the pipette solution consisted of 145 K-aspartate, 10 HEPES, 0.3 CaCl₂, and 2.2 EGTA (pH 7.1). Only cells with a resting membrane potential more negative than ~50 mV were included for study. All experiments were performed at room temperature. At the end of each experiment, neurons were tested for the presence of TTX-resistant Na⁺ current in a voltage-clamp protocol consisting of a series of 10-nV depolarizing voltage steps from ~40 to ~0 mV from a holding potential of ~80 mV. TTX (1 μM) was added to the extracellular solution with Ca²⁺ removed to eliminate interference from Ca²⁺ currents. MgTx (Alomone), a 39-amino acid peptide toxin blocker of Kv1.3 originally isolated from the venom of the scorpion Centruroides margaritatus (Garcia-Calvos et al. 1993; Knaus et al. 1995), was used in the range of 50–1,000 pM. We selected 500 pM for full block based on the concentration-response curve in the original study by Garcia-Calvos et al. (1993) and Rb⁺ flux studies in HEK or CHO cells transfected with Kv1.3 reporting IC₅₀ values of 230 and 110 pM, respectively (Koschak et al. 1998; Helms et al. 1997). To confirm the specificity of MgTx for Kv1.3 in NG neurons, DTX (10 nM) was added to the bathing solution in a subset of experiments to block Kv1.1, Kv1.2, and Kv1.6 also present in these neurons followed by 500 pM MgTx. Membrane current and voltage were recorded using a patch-clamp amplifier (Axopatch-200B, Axon Instruments, Sunnyvale, CA), digitized online (10 kHz) with an analog-to-digital interface (Digidata 1200, Axon Instruments), and filtered at 1.0 kHz. Data were analyzed using pCLAMP (version 8.0, Axon instruments).

Brain stem NTS slices. Brain stem NTS slices were prepared from animals anesthetized with 5% isoflurane and decapitated. The brainstem was removed and placed in ice-cold low-Ca²⁺/high-Mg²⁺ artificial cerebral spinal fluid (aCSF) containing the following (in mM): 124 NaCl, 3 KCl, 1.2 NaH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11 glucose, 0.4 l-aspartic acid, 2 MgCl₂, and 1 CaCl₂ saturated with 95% O₂-5% CO₂ (pH 7.4, 300 mosM). Horizontal slices (~290 μm) were cut with a vibrating microtome (Leica VT 1000S). The submerged sections were secured with a nylon mesh and superfused at a flow rate of 3–4 ml/min with standard recording aCSF [containing (in mM): 114 NaCl, 3 KCl, 1.2 NaH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11 glucose, 0.4 l-aspartic acid, 2 CaCl₂, saturated with 95% O₂-5% CO₂ (pH 7.4, 300 mosM)]. Horizontal slices (~290 μm) were cut with a vibrating microtome (Leica VT 1000S). The submersed sections were secured with a nylon mesh and superfused at a flow rate of 3–4 ml/min with standard recording aCSF [containing (in mM): 114 NaCl, 3 KCl, 1.2 NaH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11 glucose, 0.4 l-aspartic acid, 2 CaCl₂, saturated with 95% O₂-5% CO₂ (pH 7.4, 300 mosM)].

Data analysis. Second-order NTS neurons were identified by jitter analysis, defined as the SD of the latency (Doyle and Arensen 2001). Neurons with jitter values of <250 μs were considered to be mono-synaptic and directly connected to sensory TS neurons. In protocols involving a 20-Hz stimulus train, the percentage of synaptic depression in evoked current from the first event was determined. EPSC data points for a given trial were an average of five to eight EPSC sweeps at 20 Hz. Statistical analysis was performed with SigmaStat (version 3.5, Systat Software, San Jose, CA) or Origin software (Origin Labs). All data are presented as means ± SE. Electrophysiological data were compared by Student’s t-test and two-way repeated-measures ANOVA. Cumulative probability plots of spontaneous EPSCs were compared by a Kolmogorov-Smirnov two-sample test (SPSS).

Results

Kv1.3 is expressed in the NG. We examined the NG for the presence of the Kv1.3 channel. cDNA of the expected size, 792 bp, was amplified in samples of poly-(A⁺)-RNA isolated from the nodose and brain. Samples run in parallel without reverse transcriptase gave no amplification (Fig. 1A). Western blots confirmed the protein expression of Kv1.3 in the NG. The anti-Kv1.3 antibody (Alomone) recognized a prominent band with the apparent molecular mass of ~65 kDa in both postnatal day 1 and 36 tissue (Fig. 1B). We next explored the distribution of the Kv1.3 α-subunit in the soma of the NG. Kv1.3 immunostaining of the NG was present throughout the neuronal population and strongly labeled internal organelles (Fig. 2A). Immunoreactivity was weaker at/near the plasma membrane (Fig. 2A, inset). Ninety-six percent of the neurons (3,339/3,475) counted in one ganglion were labeled with anti-Kv1.3. Small, medium, and large neurons labeled with anti-Kv1.3 (Fig. 2B). Myelinated axons within the ganglion identified with anti-MBP labeled with anti-Kv1.3 (Fig. 2, D–F). Axons lightly labeled with anti-Kv1.3 but unlabeled with anti-MBP can also be seen in this image coursing parallel to the myelinated fibers. In cross sections of the ADN afferent sensory nerve with soma in the NG, Kv1.3 was identified in MBP-immunoreactive axons as well as clusters of presumed MBP-immunonegative axons (Fig. 2C).

Kv1.3 immunoreactivity is present in the presynaptic terminals in the NTS. Primary afferent fibers from visceral sensory fibers enter the brain stem NTS as part of the TS and, after exiting the tract, form a synapse with cells in this nucleus. We obtained Kv1.3 immunostaining within the tract and in the medial and commissural nuclei regions of the NTS where afferents from arterial baroreceptors and chemoreceptors terminate (Fig. 3, A and B). Much of this was punctate labeling surrounding the neurons close to the tract. To demonstrate localization to presynaptic terminals, the NTS region was labeled with both anti-Kv1.3 and anti-vesicular glutamate transporter 2, the primary glutamate vesicular transporter in the NTS (Lachamp et al. 2006) (Fig. 3, C–E). Colabeling was observed in structures of ~2 μm in diameter, consistent with our previously reported size of presynaptic terminals on NTS soma (Drewe et al. 1988). Anti-Kv1.3 immunoreactivity was not present in neuron cell bodies in the region near the tract.

MgTx blocks a component of the somal outward K⁺ current. We next asked whether Kv1.3 is expressed in the surface membrane of the NG soma using a functional assay. We isolated whole cell K⁺ currents from Na⁺ and Ca²⁺ currents by replacing Na⁺ in the external solution with equimolar...
NMDG\(^+\) and reducing extracellular Ca\(^{2+}\) to 0.03 mM. In initial experiments, a depolarizing voltage step to +40 mV was applied at 20- to 30-s intervals from a holding potential of −80 mV. When the outward K\(^+\) current was stable for at least 2 min, 500 pM MgTx was added to the bath solution to block Kv1.3 (Garcia-Calvo et al. 1993; Helms et al. 1997) (Fig. 4A). As shown in Fig. 4, MgTx blocked a portion of the whole cell K\(^+\) current. In eight of eight neurons, the current decreased: 14.0 ± 3.8 pA/pF (range: 3–33 pA/pF) at the peak and 8.4 ± 2.2 pA/pF (range: 4–21 pA/pF) at the end of the 100- to 150-ms step. The former corresponded to 10.1 ± 1.8% and the latter to 5.6 ± 1.1% of the total K\(^+\) current at those time points. The inset shows the time course of the MgTx block. Increasing the concentration of MgTx to 1.0 nM had no further effect (n = 3). In a second set of experiments, a 900-ms ramp stimulus delivered every 30 s from −100 to +80 mV was used to obtain a current-voltage relationship, which showed the MgTx-sensitive current activated more positive than −30 mV (Fig. 4B). Under current clamp, we observed no effect on the resting membrane potential at the addition of MgTx (0.5–1 nM) (control: −62.0 ± 1.7 mV vs. MgTx: −61.8 ± 1.6 mV, n = 23, P > 0.3, paired t-test), consistent with the lack of MgTx block under voltage clamp at potentials in this voltage range.

MgTx alters action potential duration in C-type but not A-type neurons. Somal recordings are used to gain insight into function in regions that are less accessible, such as the central presynaptic terminal. In the present study, we focused on the contribution of Kv1.3 to action potential duration under current-clamp conditions as an indicator of a potential role in central transmitter release. The natural stimulus received by sensory neurons from their peripheral terminals is a series of action potentials whose frequency is dependent on stimulus intensity at the peripheral receptor terminal. Thus, an appropriate stimulus to evaluate the role of Kv1.3 is a short-duration stimulus designed to elicit single action potentials. Neurons were stimulated at 0.5 Hz (0.35–1.5 ms) in current-clamp mode. When MgTx was applied to the rare cells subsequently shown to express only TTX-sensitive Na\(^+\) current (A-type neurons) (Stansfeld and Wallis 1985; Schild and Kunze 1997; Li and Schild 2007b), there was no change in the duration of the action potential measured at 0 mV (0.74 ± 0.09 vs. 0.76 ± 0.1 ms, n = 3, P > 0.4, paired t-test). In addition, in three neurons, there were no changes in the duration of the action potential measured at 20 mV.

**Fig. 2.** Immunohistochemical localization of Kv1.3 in the NG and aortic depressor nerve (ADN). A: anti-Kv1.3 (Neuromab) immunolabeling in postnatal day 30 Sprague-Dawley rat nodose slices. The image is a mass z-projection of five confocal sections acquired at 0.69-μm intervals. Calibration bar = 30 μm. The inset shows a zoomed image to illustrate weaker labeling near/at the membrane. B: histograms showing the broad distribution of anti-Kv1.3 labeling with the respective neuron diameter compared with the distribution of diameters in the total population. C: section of the ADN colabeled with anti-Kv1.3 (Alomone; red) and anti-myelin basic protein (MBP; green) to illustrate the presence of Kv1.3 in myelinated axons. Arrows indicate examples of the anti-Kv1.3 label. These results were obtained in the ADN from two animals. Note the presence of only two large-diameter axons. Similar results (2–3 large axons with diameters > 2 μm) were obtained in five other ADNs. Scale bar = 2 μm. D and E: images of 8-μm nodose sections colabeled with anti-Kv1.3 (red; D) and anti-MBP (green; E). The overlay (F) shows the presence of Kv1.3 antibody in myelinated and unmyelinated axons in the ganglion. Scale bar = 20 μm.
potential in response to 20-Hz repetitive stimulation either in the control solution or in the presence of MgTx (Fig. 5, A and B). To verify that Kv1.3 was, however, functionally expressed in these neurons, we applied a longer-duration constant current depolarizing stimulus (150 or 400 ms) at a threshold level for eliciting at least one action potential. Application of MgTx either increased the number of action potentials in response to the stimulus (2 of 3 neurons; Fig. 5A, top inset) and/or increased the amount of depolarization produced by the current injection (1 of 3 neurons; Fig. 5A, bottom inset), with the latter indicative of a decrease in membrane conductance upon block of Kv1.3.

A second group of neurons (n = 6) with intermediate-duration action potentials did not respond to MgTx (action potential duration: 1.64 ± 0.12 vs. 1.65 ± 0.11 ms in MgTx, n = 6, P > 0.05) when stimulated at 0.5 Hz and showed no change in duration at 20 Hz (P > 0.05, paired t-test; Fig. 5B). The Na⁺ current in this group was incompletely blocked by TTX. We considered these to represent the Ah class of neurons with more slowly conducting myelinated fibers and an action potential duration of >1.0 ms, as described by Stansfeld and Wallis (1985) and, more recently, by Li et al. (2007b). As with the previous group, these Ah-type neurons responded to MgTx during a sustained depolarization with an increase in the number of action potentials to the current injection (4 of 7 cells) or a larger depolarization (3 of 7 cells, range: 2–5 mV).

The last group of NG neurons, C-type neurons, have broader initial action potentials (>2.0 ms) and express TTX-resistant current. These C-type responded to MgTx with an increase in the duration of the action potential from 2.19 ± 0.24 to 2.31 ± 0.23 ms at 0 mV (n = 13, P < 0.01, paired t-test) in response to a 0.5-Hz stimulus. This group was also subjected to a 20-Hz stimulation protocol in the absence and presence of MgTx (Fig. 5, C and D). MgTx has shown a high selectivity for Kv1.3 over other K⁺ channels, such as Ca²⁺-activated K⁺ channels (Leonard et al. 1992) and Kv3.1, Kv1.5, and IsK channels (Calvogarcia et al. 1993). The latter group reported has a weak effect on Kv1.6. Comparable studies have not been done for Kv1.1 and Kv1.2, which are both present in nodose neurons (Glazebrook et al. 2002). Thus, before the addition of MgTx, four C-type neurons were incubated with DTX (50 nM) to block Kv1.1, Kv1.2, and Kv1.6 currents. While this increased the duration of their action potentials, as expected (Glazebrook et al. 2002), all four neurons subsequently responded to MgTx with a further increase in action potential duration and are included in the grouped 20-Hz data (Fig. 5D). The MgTx effect...
was superimposed on an increase in action potential duration in response to 20-Hz repetitive stimuli (Fig. 5, C and D), a characteristic of C-type neurons, as previously reported by Li and Schild (2007a).

*MgTx augments synaptic transmission in the NTS.* We asked whether the inhibition of Kv1.3 by MgTx observed in sensory afferents alters central neurotransmitter release in NTS brain slices. We recorded EPSCs from 18 NTS neurons in the medial and commissural NTS within the region of Kv1.3 immunoreactivity. EPSC synaptic latency was 3.7 ± 0.4 ms, and its SD (i.e., jitter) was 135 ± 14 μs, suggesting that EPSCs were generated from a monosynaptic connection (Doyle and Andreassen 2001). Overall, the initial event of a 20-Hz EPSC train in the control solution ranged from 48 to 681 pA, with 15 of 18 cells exhibiting amplitudes of <300 pA (average: 137 ± 19 pA) with the remainder of cells averaging 536 ± 91 pA (3 of 18 cells, P < 0.05 vs. smaller events). The neurons with smaller EPSCs exhibited sensitivity to MgTx compared with those that exhibited larger initial EPSCs. A representative example of a MgTx-sensitive NTS cell is shown in Fig. 6A. In this cell, MgTx increased the amplitude of TS-evoked EPSCs that were elicited at 20 Hz. Mean data for the group of 15 smaller event, MgTx-sensitive cells was shown in Fig. 6B. Bath application of MgTx (20 nM, 6–7 min) increased the amplitude of the initial TS-evoked EPSCs. The amplitude of the first EPSC averaged 137 ± 19 pA in control recordings and increased to 187 ± 26 pA in MgTx (P = 0.016, paired t-test). Across the TS-EPSC event train, the MgTx-sensitive TS-EPSC amplitude was significantly greater at the beginning of the train and reduced at the end (two-way repeated-measures ANOVA; Fig. 6B). Spontaneous EPSCs in these 15 smaller event, MgTx-sensitive cells were also evaluated. Cumulative probability plots of spontaneous EPSC amplitudes and interevent intervals were generated to analyze distribution. MgTx did not alter the amplitude of spontaneous events (P = 0.295, Kolmogorov-Smirnov test; Fig. 6C, left) but did reduce the interevent interval, indicating a small increase in event frequency (P = 0.02, Kolmogorov-Smirnov test; Fig. 6C, right). The second group of larger EPSC amplitude monosynaptic neurons did not respond to MgTx (Fig. 6D). Note that the amplitudes of TS-EPSCs that were sensitive to MgTx were significantly smaller across the stimulus train than MgTx-insensitive NTS neurons (two-way repeated-measures ANOVA).

**DISCUSSION**

In this study, we show a functional role for Kv1.3 at the first synapse in visceral afferent pathways in the NTS. Our results.
support a role for Kv1.3 modulating transmitter release from presynaptic terminals of C-fibers through effects on the duration of the action potential.

*Kv1.3 as a presynaptic modulator of transmitter release.* Consistent with reports of an axonal distribution for Kv1.3 (Veh et al. 2005; Rivera et al. 2005), we found immunoreactivity in the central axonal branches in the tract extending to presynaptic terminals. Localization of anti-Kv1.3 with vesicular glutamate transporter 2 along with the fact that Kv1.3 immunoreactivity was not present in NTS neurons in this region supports a presynaptic site for the actions of MgTx. Presynaptic localization has also been reported recently in the calyx of Held (Gazula et al. 2010).

*Kv1.3 modulates transmitter release at presynaptic terminals.* We assessed the role of Kv1.3 at the central terminals by monitoring EPSCs in second-order NTS neurons in the medial and commissural NTS receiving monosynaptic sensory input. In response to tract stimulation, the EPSC amplitude was increased in the presence of MgTx in a subset of neurons. Extrapolating from our data in the nodose soma, where the C-fiber population (but not A- or Ah-type neurons) responds to MgTx with an increase in the duration of the action potential, we propose that the responding NTS neurons are innervated by C-type fibers and that the increase in the duration of the presynaptic action potential in the presence of MgTx augments transmitter release, leading to an increased amplitude of the synaptic potential (Wheeler et al. 1996; Raffaelli et al. 2004; Stephens and Mochida 2005). A role for Kv1.3 in transmitter release has been reported in other neuronal studies (Ohno-Shosaku et al. 1996; Shoudai et al. 2007; Doczi et al. 2008).

Our data suggest that Kv1.3 in presynaptic terminals, presumably from C-type fibers, plays a significant role in modulating neurotransmitter release at the NTS. A second group of EPSCs in NTS neurons was not altered by MgTx. This is consistent with innervation by fibers from A- and Ah-type neurons with short duration action potentials, where Kv1.3 would not play a role unless there were sustained depolarization of the terminal. We cannot rule out, however, the possibility that Kv1.3 is not present in the presynaptic terminals of the myelinated fibers, although it is present in their soma and axons. Interestingly, the evoked EPSCs that were not altered by MgTx were significantly larger in amplitude than those that were. Data from Andresen and Peters (2008) support the conclusion that the larger nonresponsive EPSCs in our study were present in neurons innervated by A- or Ah-type fibers. Those authors compared the peak amplitudes of capsaicin-resistant tract evoked EPSCs, presumably innervated by myelinated fibers, with capsaicin-sensitive EPSCs, innervated by C-type unmyelinated fibers. The former were 50% larger than the latter.

Fig. 6. MgTx augments TS-evoked excitatory postsynaptic currents (EPSCs). A: representative tracings of TS evoked EPSCs that were recorded under control conditions (in artificial cerebrospinal fluid) and after MgTx (20 nM). The TS was stimulated at 20 Hz. Note the increase in the TS-EPSC amplitude, especially the first event. Shown is an average of five current sweeps.

B: synaptic events were grouped according to their initial current amplitude. Data shown are the mean TS-EPSC amplitudes for 20 events whose initial amplitudes were <300 pA during control and MgTx application. n = 15. *P < 0.05 (two-way repeated-measures ANOVA). MgTx elevated EPSCs primarily at the beginning of the stimulus train. C: spontaneous EPSC frequency was also elevated in MgTx-sensitive currents. The cumulative probability of sEPSC amplitude distribution (2-pA bin, left) was not altered in MgTx. On the other hand, the cumulative fraction of spontaneous EPSC interevent intervals (10-ms bin) illustrated a small leftward shift in the presence of MgTx (right). The analysis was performed on the entire sample of events.

D: mean TS-EPSC amplitude for 20 events during control and MgTx application for three cells whose initial TS-EPSCs were >300 pA and did not respond to MgTx application. Note that the amplitudes of the EPSCs that were sensitive to MgTx (B) were significantly smaller than those of MgTx-insensitive NTS neurons.
Frequency-dependent depression persists in the presence of MgTx. Frequency dependent depression (FDD) is a hallmark characteristic of the NTS synapse, was first described by Miles (1986), and is readily demonstrated the results shown in Fig. 6. FDD has been proposed to result from a reduction in the availability of a readily releasable pool of vesicles at the presynaptic site (Schild et al. 1995), and it remains even in the presence of MgTx (Fig. 6). Superimposed on FDD is another factor that could potentially alter transmitter release during repetitive stimulation. This arises from the presence of presynaptic K⁺ channels that would limit action potential duration but that undergo cumulative inactivation in response to stimulation. These include the Kv1.3 channel (Cahalan et al. 1985; Maron and Levitan 1994, Grissmer et al. 1994), the large-conductance Ca²⁺-activated K⁺ channel KCNMA1 (Shao et al. 1999), and the Kv2.1 channel (Klemic et al. 1998), all channels that are present in the NG. One would expect that the broader action potentials toward the end of a stimulus train would lead to an increase in transmitter release. However, it appears that as FDD develops at the central synapse, it over-rides the postulated effect of the broader action potentials that we observed during the 20-Hz stimulation in the soma of C-type neurons.

Physiological significance of presynaptic Kv1.3 in C-type axons. At the presynaptic site in the NTS, most of the effect of Kv1.3 on transmitter release would occur early in a stimulus train before FDD develops. In our study, MgTx was most effective on the amplitude of the ESPC in response to the first stimuli in the 20-Hz series, where the hyperpolarizing effect of Kv1.3 is expected to play a stronger role at a time when competing FDD is still developing. Miles (1986) reported that the amplitude of the EPSC was reduced to 91% of its control value at a stimulation frequency of 1 Hz, to 63% at 5 Hz and 39% at 10 Hz. Thus, at low frequencies, there is minimum depression. This is important because the visceral sensory C-fiber population tends to fire at low frequencies (<1–5 Hz). For instance, C-fiber chemoreceptors generally fire from ≤ 2 to 4–5 Hz, although at very strong stimulation of their sensory terminals discharge can reach 20 Hz (Sato and Fidone 1969). The same study showed arterial baroreceptor C-fibers responded to a natural stimulus with only 1–2 spikes per arterial pressure pulse at a rate of 2–3 Hz, although under extreme stimulus conditions this can reach 15–20 Hz. Other studies have confirmed these results (Coleridge et al. 1987; Seaard et al. 1990; Thoren et al. 1999).

Conclusions. In summary, this is the first study to implicate a specific K⁺ channel, Kv1.3, in the evoked release of transmitter at second-order neurons receiving sensory input in the NTS. The data support the contention that Kv1.3 plays a role in limiting the duration of C-fiber action potentials, which is most effective during brief bursts of two to three action potentials with minimal cumulative inactivation and FDD, well within the range of the in vivo activity of C-fiber populations.

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