Kv1.3 channels in postganglionic sympathetic neurons: expression, function, and modulation

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Doczi MA, Morielli AD, Damon DH. Kv1.3 channels in postganglionic sympathetic neurons: expression, function, and modulation. Am J Physiol Regul Integr Comp Physiol 295: R733–R740, 2008. First published July 9, 2008; doi:10.1152/ajpregu.00077.2008.—Kv1.3 channels are known to modulate many aspects of neuronal function. We tested the hypothesis that Kv1.3 modulates the function of postganglionic sympathetic neurons. RT-PCR, immunoblot, and immunohistochemical analyses indicated that Kv1.3 channels were expressed in these neurons. Immunohistochemical analyses indicated that Kv1.3 protein was localized to neuronal cell bodies, processes, and nerve fibers at sympathetic neurovascular junctions. Margatoxin (MgTX), a specific inhibitor of Kv1.3, was used to assess the function of the channel. Electrophysiological analyses indicated that MgTX significantly reduced outward currents \( P < 0.05; n = 18 \) (control) and 15 (MgTX)), depolarized resting membrane potential, and decreased the latency to action potential firing \( P < 0.05; n = 11 \) (control) and 13 (MgTX)). The primary physiological input to postganglionic sympathetic neurons is ACh, which activates nicotinic and muscarinic ACh receptors. MgTX modulated nicotinic ACh receptor agonist-induced norepinephrine release \( P < 0.05; n \geq 6 \), and MgTX-sensitive current was suppressed upon activation of muscarinic ACh receptors with bethanechol \( P < 0.05; n = 12 \). These data indicate that Kv1.3 affects the function of postganglionic sympathetic neurons, which suggests that Kv1.3 influences sympathetic control of cardiovascular function. Our data also indicate that modulation of Kv1.3 is likely to affect sympathetic control of cardiovascular function.

The sympathetic nervous system is a major determinant of cardiovascular function and is implicated in cardiovascular disease (2–4, 7, 9, 15, 23, 24, 29, 32, 40, 41). Many effects of the sympathetic nervous system on cardiovascular function are mediated via neurotransmitters released from postganglionic sympathetic neurons innervating blood vessels. The mechanisms governing neurotransmitter release at sympathetic neurovascular junctions are not completely understood.

Kv1 family potassium channels consist of eight genes encoding distinct alpha subunit proteins, Kv1.1 through Kv1.8, and are expressed throughout the nervous system (5, 13, 14, 18, 20, 33, 37, 39, 43, 44, 46, 48). Functional Kv1 channels are formed when four Kv1 alpha subunits assemble as homotetramers or as heterotetramers with other members of the Kv1 family. These channels affect a range of neuronal functions (6, 8, 11, 16, 26, 34–36), including spike frequency adaptation (35, 36) and the regulation of cellular excitability in response to synaptic input (11). Postganglionic sympathetic neurons express Kv1 channels (5, 33, 43), and inhibition of these channels has been reported to modulate neurotransmitter release from these neurons (21, 22, 45). However, the mechanisms involved in Kv1 channel modulation of neurotransmitter release in sympathetic neurons are largely unexplored.

Kv1.3 channels play a key role in a wide range of physiologic phenomena. Kv1.3 is required for the activation of T-lymphocytes and is thus a determinant of immune function (1). Inhibition of Kv1.3 facilitates translocation of the insulin-sensitive glucose transporter, GLUT4, to the plasma membrane of adipocytes (31) and skeletal muscle (50), and is thus a determinant of glucose homeostasis. Kv1.3 has also been shown to contribute to body weight regulation and energy homeostasis, processes that are regulated by the sympathetic nervous system (49). In neurons, Kv1.3 has been reported to modulate action potential firing (8, 26).

Here, we evaluate the role of Kv1.3 in postganglionic sympathetic neuron function. We demonstrate that this channel is present and functional in postganglionic sympathetic neurons. Kv1.3 was detected throughout the neurons, in both soma and processes. It contributed to outward currents recorded from the soma and was a determinant of resting membrane potential and neurotransmitter release. These studies show that Kv1.3 channels are important determinants of postganglionic sympathetic neuronal function and have important implications for understanding the effects of the sympathetic nervous system on cardiovascular function.

METHODS

Animals. The use of animals in the present studies was in accordance with the National Institutes of Health guidelines for the humane care and use of animals in research and was approved by the Institutional Animal Care and Use Committee of the University of Vermont. Neonatal Sprague-Dawley rats were used to obtain superior cervical sympathetic ganglia (SCG). Adult postpartum female Sprague-
Dawley rats were used to obtain SCG and tail arteries. The postpartum females used in the present studies were the mothers of the neonatal rats and were used to minimize the number of animals.

**Neuronal culture.** Postganglionic sympathetic neurons were isolated from the SCG of neonatal (3 or 4 days) Sprague-Dawley rats (males and females). Ganglia were dissociated for 10 min at 37°C in a collagenase/hyaluronidase solution (10 mg/ml BSA, 4 mg/ml collagenase, 1 mg/ml hyaluronidase in Dulbecco’s PBS) and then for 10 min in trypsin (3 mg/ml added to trypsin-EDTA). Dissociated cells were resuspended in neuronal growth medium [DMEM/F12 supplemented with 10% NuSerum (BD Biosciences), 5% fetal bovine serum (Invitrogen) and penicillin/streptomycin], supplemented with NFG (BD Biosciences; 50 ng/ml), and applied to collagen-coated tissue culture dishes. The cells were allowed to attach overnight in a humidified 5% CO2 environment maintained at 37°C. Nonneuronal cells were then growth arrested with mitomycin C (Sigma; 10 ng/ml) containing 3% nonfat dry milk, and primary antibody (0.2 μg/ml) containing 0.05% Tween and 3% nonfat dry milk (30 min at room temperature). 4–20% gradient acrylamide gels. Samples were then transferred to Western blot running buffer, boiled for 5 min, and electrophoresed on the gel. Products were sequenced by the University of Vermont DNA facility and equal amounts of cDNA amplified (Amplitaq Gold, Applied Biosystems). PCR primers and annealing temperatures are indicated in Table 1. PCR products were electrophoresed on 1.2% agarose gels containing ethidium bromide and visualized with UV light. All PCR reactions included (−) RT and (−) template controls. Amplified PCR products were sequenced by the University of Vermont DNA facility to confirm the identity of the DNA.

**Western blot analyses.** Tissues and cells were lysed and homogenized in enhanced RIPA buffer [50 mM Tris, 150 mM NaCl, 10 mM EDTA 0.2% deoxycholate 1% NP40, 10% glycerol, 1% protease inhibitor cocktail (Sigma), 1 mM dithiothreitol, 0.1% sodium dodecyl sulfate; pH 8.0]. Samples were diluted with equal volumes of electrophoresis running buffer, boiled for 5 min, and electrophoresed on 4–20% gradient acrylamide gels. Samples were then transferred to nitrocellulose membranes. The membranes were blocked with PBS containing 0.05% Tween and 3% nonfat dry milk (30 min at room temperature) and then incubated overnight at 4°C in PBS-Tween containing 3% nonfat dry milk, and primary antibody (0.2 μg/ml anti-Kv1.3 (NeuroMab) and 1 μg/ml anti-tyrosine hydroxylase (TH; Sigma)). The Kv1.3 mouse monoclonal antibody, IgG2a isotype, was raised against synthetic peptide amino acids 485-506 of rat Kv1.3 (clone L23/27; Lot # 413-5RR-07). The TH mouse monoclonal antibody recognizes the N-terminal epitope between amino acids 40–48 of TH (clone L23/27; Lot # 413-5RR-07). Unbound primary antibody was removed with three 5-min washes (PBS-Tween). The membranes were then incubated in PBS-Tween containing 3% nonfat dry milk and horseradish peroxidase-conjugated secondary antibodies (1:3,000; Bio-Rad) for 1 h at room temperature. The horseradish peroxidase was detected with enhanced chemiluminescence (Pierce) and documented on autoradiographic film.

**Electrophysiology.** Electrophysiological recordings were performed at room temperature, using the whole cell patch-clamp technique. Data acquisition and analysis were obtained using the Axopatch 200B (Axon Instruments) patch-clamp amplifier and pCLAMP 9.2 (Axon Instruments) software. Electrodes were pulled in two stages from thin-wall filament glass capillary tubing (Warner Instruments) and fire polished to a resistance ranging from 1 to 2 MΩ. Voltage-clamp recording solutions were as follows (in mM): external (bath) solution 100 NaCl, 5.4 KCl, 1.8 CaCl2, 0.8 MgCl2, 23 glucose, 5 Na HEPES, 0.001 TTX, 10 tetraethylammonium (TEA), pH 7.4; internal (pipette) solution: 120 KCl, 1.8 CaCl2, 0.8 MgCl2, 23 glucose, 5 Na HEPES, 0.001 TTX, 10 tetraethylammonium (TEA), pH 7.4; internal (pipette) solution: 120 KCl, 3.69 CaCl2, 0.094 MgCl2, 5 BAPTA, 5 EDTA, 5 Na HEPES, 5 glucose, pH 7.2. Pharmacological agents were applied at the following concentrations: 1 mM mGTX (MQTX; Alomone Labs), 100 mM α-dendrotoxin (DTX; Research Biochemicals International), and 100 μM bethanecol (BeCh; Sigma). Cells were held at −60 mV, followed by a 20-ms hyperpolarization to −90 mV, and stepped from −70 mV to +50 mV in 10-mV increments. Leak currents (P/L) were subtracted from all traces. Averaged MgTX traces were subtracted from averaged control traces to obtain the resolved Kv1.3 current. Current clamp recording solutions were the same as listed above, except that TTX and TEA were omitted. Resting membrane potential was monitored for 100 ms, followed by 400-ms current injections of the following magnitudes (in pA): −500, 500, 1,000, 1,500, 2,000, and 2,500. Latency to action potential firing was defined as the time between the start of the current injection and the peak of the action potential. Action potential width was measured at half the action potential amplitude as previously described (12).

**Norepinephrine release.** Norepinephrine (NE) release was assessed using tritiated norepinephrine purchased from Amersham. These assays were performed using HEPES-buffered Krebs solution [122 mM NaCl, 3 mM KCl, 0.4 mM MgSO4·H2O, 1.2 mM KH2PO4, 10 mM glucose, 20 mM HEPES, 1.3 mM CaCl2·2H2O, 1 mM ascorbic acid, 10 μM pargyline, pH 7.4]. Cells were preincubated at 37°C with 100-nM tritiated norepinephrine for 30 min. The cells were then washed (6 × 5 min) and stimulated with a nicotine agonist, dimethylphenylpiperazinum (DMP; 30 μM; Sigma). The remaining cell-associated NE was then extracted with acidified ethanol. NE in all samples was collected and analyzed using a Beckman LS6000IC Liquid Scintillation Counter (Beckman Instruments). Stimulated-released

### Table 1. Forward and reverse PCR primer sequences

<table>
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<th>mRNA</th>
<th>Primer Sequences</th>
<th>Annealing Temperature, °C</th>
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<tr>
<td>Kv1.3</td>
<td>5′-GTA CCG ACG CCC GCT GCA TGA-3′</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>5′-GGG CAA GGA AAG AAT CCC AGC-3′</td>
<td>60</td>
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**Neuronal transfection.** Neurons were transfected using the Helios Gene Gun (Bio-Rad) with pEGFP or pEGFP-Kv1.3. pEGFP-coated bullets were a generous gift from Dr. Victor May (University of Vermont, Department of Anatomy and Neurobiology). pEGFP-C1-Kv1.3 was a generous gift from Dr. Jürgen Kupper [25; Max Planck Institute of Biochemistry, Martinsried, Germany]. Cells were studied 48 h after transfection.

The same monoclonal Kv1.3 antibody (0.84 μg/ml; NeuroMab) used for Western blot analyses was also used to identify Kv1.3. Cells were incubated in primary antibodies overnight at 4°C, followed by three 5-min washes in PBS. Alexa Fluor Goat anti-mouse (cultures 568 nm; SCG and tail arteries 647 nm) and goat anti-rabbit (488 nm) secondary antibodies (4 μg/ml; Invitrogen) were applied for 1 h at room temperature, and all samples were mounted using ProLong Gold antifade reagent (Invitrogen). All images were taken using the Olympus IX70 microscope and DeltaVision Restoration Imaging System (Applied Precision, LLC) and background subtracted with an IgG2a isotype control (R&D Systems).
lease was calculated using the following equation: (stimulated cpm – background cpm)/(total cpm available for release).

**Statistics.** Data are presented as means ± SE. Unpaired Student’s t-tests assuming unequal variances were used to determine statistical differences. Differences were considered significant if $P \leq 0.05$.

**RESULTS**

To begin to assess the function of Kv1.3 channels in postganglionic sympathetic neurons, we first characterized the expression and subcellular localization of these channels. Kv1.3 mRNA and protein were detected in dissociated neurons (d) and in intact neonatal (n) and adult (a) ganglia (Fig. 1A). Potassium channel effects on neuronal physiology are strongly influenced by their location within the cell; therefore, we used immunofluorescence microscopy to examine the subcellular localization of Kv1.3 protein (Fig. 1, B and C). Neurons were identified with antibodies directed against TH. In dissociated neurons and freshly isolated tissues, Kv1.3 was detected in both the soma and processes. In the soma, Kv1.3 exhibited a striking pattern of localization to a discrete intracellular compartment that overlaps with the GM130 Golgi apparatus marker in dissociated neurons (Fig. 2). All dissociated neurons observed expressed Kv1.3 and exhibited this intracellular localization pattern.

Because the striking intracellular localization detected with anti-Kv1.3 is not typical for a membrane-associated ion channel, we used GFP-Kv1.3 as an alternative approach to assess the subcellular localization of Kv1.3. The distribution of overexpressed GFP-Kv1.3 overlapped with that detected by Kv1.3 immunofluorescence (Fig. 3). The distribution of overexpressed GFP differed considerably from GFP-Kv1.3 and Kv1.3 immunofluorescence (Fig. 3). Intracellular compartmentalization of Kv1.3 was also observed in sympathetic neurons in adult ganglia (Fig. 1C).

We next determined the function of Kv1.3 in postganglionic sympathetic neurons. We used whole cell patch-clamp electrophysiology in the absence and presence of MgTX, a specific inhibitor of Kv1.3 (10). To resolve Kv1.3 currents, all measurements were recorded in the presence of 1 μM TTX and 10 mM TEA to block currents generated by sodium and non-Kv1 family potassium channels. Steady-state currents measured in untransfected dissociated postganglionic sympathetic neurons were significantly suppressed by MgTX (1 nM; $n = 15$) relative to control ($n = 18; P < 0.02$) (Fig. 4A). This indicates that endogenous Kv1.3 channels contribute to outward current recorded from the soma of postganglionic sympathetic neurons. To confirm that MgTX inhibited Kv1.3, steady-state currents were also measured in dissociated postganglionic sympathetic neurons expressing GFP-Kv1.3. Outward currents in transfected cells were markedly increased, indicating that GFP-Kv1.3 was functional in these cells. MgTX (1 nM) elicited an 86% decrease in this current (Fig. 4B; $n = 3; P < 0.002$). Activation curves were generated from tail currents and fit to a Boltzmann function. These curves are shown in Fig. 4C for both endogenous and GFP-Kv1.3. Half-activation voltages ($V_{1/2}$) determined from these curves were $-22.8$ mV for endogenous MgTX-sensitive current and $-27.2$ mV for GFP-Kv1.3, values consistent with previously published reports (47).

At concentrations higher than that used in the present studies, MgTX has been reported to inhibit Kv1.6 (10). To evaluate the contribution of Kv1.6 to the MgTX-sensitive current measured in Fig. 4A, we measured steady-state ionic currents in dissociated postganglionic sympathetic neurons in the presence of DTX, an inhibitor of Kv1.6, as well as Kv1.1 and Kv1.2 channels (17). DTX (100 nM) had no effect on steady-state currents in these cells ($P > 0.05; n \geq 10$) (Fig. 4D). Steady-state outward currents recorded in HEK 293 cells stably transfected with Kv1.2 were significantly suppressed in the presence of DTX (100 nM; $n = 4; P < 0.05$; data not shown), confirming the effectiveness of this inhibitor. These findings support our conclusion that the MgTX-sensitive current shown in Fig. 4A is attributable to Kv1.3.

Current clamp electrophysiology was used to elucidate the physiological role of Kv1.3 current in dissociated postganglionic sympathetic neurons. Resting membrane potential measured in the presence of MgTX ($-45.7$ mV ± 2.1; $n = 13$) was significantly depolarized relative to control ($-51.9$ mV ± 2.3; $n = 11; P < 0.05$) (Fig. 5B). In addition, the latency to action potential firing in the presence of MgTX ($26.9$ ms ± 1.3; $n = 13$) was significantly less than control ($39.9$ ms ± 5.4; $n = 11; P < 0.05$) (Fig. 5C). MgTX did not alter the width of the action potential measured at half-peak amplitude (Fig. 5D).

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Fig. 1. Kv1.3 channels are present in postganglionic sympathetic neurons. A: RT-PCR (top; $n = 2$) shows expression of mRNA for Kv1.3 in neonatal dissociated neurons (d), neonatal sympathetic superior cervical ganglia (SCG) (n) and adult sympathetic SCG (a). PCR reactions included minus reverse transcriptase (-RT) and minus template (-T) controls. Kv1.3 and tyrosine hydroxylase (TH) immunoblots (bottom; $n = 2$) show corresponding protein expression. TH was used as a marker of postganglionic sympathetic neurons. Approximate molecular weight is noted with arrows. B: Immunolocalization of TH (top) and Kv1.3 (bottom) in the soma (left) and processes (right) of dissociated postganglionic sympathetic neurons in vitro ($n = 2$). Scale bar = 10 μm. C: Immunolocalization of TH (top) and Kv1.3 (bottom) in neuronal soma of intact adult sympathetic SCG (left) and of processes innervating adult rat tail arteries (right) ($n = 2$). SCG scale bar = 10 μm; tail artery scale bar = 30 μm.
ACh is the preganglionic neurotransmitter for postganglionic sympathetic neurons. ACh activates both nicotinic (nAChR) and muscarinic (mAChR) ACh receptors in these neurons (28). Activation of mAChRs is known to suppress ionic current of Kv1 family channels (19). We found that BeCh (100 μM), a mAChR-selective agonist, significantly decreased outward current measured in dissociated neonatal sympathetic neurons (Fig. 6A; n = 12; P < 0.05). Inhibition of Kv1.3 with MgTX abrogated the effect of BeCh, suggesting that activation of mAChRs suppresses Kv1.3 current in these cells (Fig. 6B; n = 12; P > 0.05). In HEK cells lacking muscarinic receptors, but transfected with GFP-Kv1.3, BeCh did not decrease Kv1.3 current at any voltage (data not shown; n = 8; P > 0.05). These data indicate that BeCh did not directly affect Kv1.3.

The ultimate function of postganglionic neurons is to release neurotransmitters, thereby affecting the function of sympathetic targets. Therefore, we assessed the effect of Kv1.3 on NE release from these neurons. ACh is the physiological stimulus for neurotransmitter release from these neurons. This effect is primarily mediated by activation of nAChRs. Therefore, we used DMPP, a nAChR-selective agonist to stimulate neurotransmitter release. Application of 30 μM of DMPP stimulated NE release from dissociated neonatal neurons. Inhibition of Kv1.3 with MgTX (1 nM) increased NE release from these cells (Fig. 7; n = 6; P < 0.05).

DISCUSSION

The present studies provide novel evidence that Kv1.3 determines the function of postganglionic sympathetic neurons. Expression analyses indicated that Kv1.3 is present in these neurons. Electrophysiological analyses indicated that this channel contributes to outward current and the maintenance of resting membrane potential. Pharmacological inhibition of Kv1.3 increased neurotransmitter release. In addition, we demonstrated that this channel was modulated by preganglionic mechanisms. Postganglionic sympathetic neurons are important determinants of cardiovascular function. Thus, our studies strongly suggest that Kv1.3 channels and their modulation are important determinants of sympathetic control of cardiovascular function.

The present studies are the first to demonstrate that Kv1.3 protein is expressed in postganglionic sympathetic neurons. RT-PCR, immunoblot, and immunohistochemical analyses indicate that Kv1.3 was in the soma. Kv1.3 was concentrated in an intracellular compartment (Figs. 1, 2, and 3). Similar localization of Kv1.3 has been reported in other cells and tissues (13, 38). Figure 2 indicates that this compartment is the Golgi apparatus. Kv1.3 was also detected in the processes of dissociated neurons, as well as in nerve fibers on the adventitial surface of freshly isolated tail arteries. These data suggest that Kv1.3 channels are likely to affect the function of postganglionic sympathetic neurons in general and, in particular, to affect sympathetic control of vascular function.

Functional analyses of Kv1.3 indicate that this channel is a determinant of the electrophysiological properties of postganglionic sympathetic neurons. MgTX, a specific inhibitor of Kv1.3 (10), decreased outward currents measured in these cells (Fig. 4A). In contrast, DTX, an inhibitor of Kv1.6, Kv1.1, and Kv1.2 (17), had no effect on outward currents in these neurons (Fig. 4D). This indicates that MgTX is specific for Kv1.3 and that Kv1.1, Kv1.2, and Kv1.6 do not significantly contribute to the outward current measured in these neurons. Action potential analyses presented in Fig. 5 indicate that Kv1.3 contributes to the maintenance of resting membrane potential. MgTX significantly decreased the latency to action potential firing, consistent with the depolarization of resting membrane potential. This is the first demonstration that Kv1.3 affects the function of postganglionic sympathetic neurons.

Our immunohistochemical and functional analyses indicate that Kv1.3 is localized to both the plasma membrane and Golgi apparatus in postganglionic sympathetic neurons. We detected

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Fig. 2. Kv1.3 overlaps with a marker for the Golgi apparatus in postganglionic sympathetic neurons. Immunoreactivity of endogenous Kv1.3 (red) overlaps with the Golgi marker, GM130 (green), in the soma of dissociated postganglionic sympathetic neurons. Right: overlap between these two antibodies (merge; n = 3). Scale bar = 10 μm.

Fig. 3. Localization of transfected GFP-Kv1.3 vs. endogenous Kv1.3 in postganglionic sympathetic neurons. GFP-Kv1.3 (top right) and immunoreactive Kv1.3 (top right) overlap in postganglionic sympathetic neurons (n = 3). Neurons transfected with GFP only (bottom left) do not overlap with immunoreactive Kv1.3 (bottom right) (n = 3). Scale bar = 10 μm.
Fig. 4. Voltage-clamp analyses of Kv1.3 in postganglionic sympathetic neurons. A: ionic current measured in untransfected dissociated sympathetic neurons in the absence (control; \( n = 18 \)) and presence of 1 nM margatoxin (MgTX; \( n = 15 \)). Current traces for each condition represent the average of unpaired measurements made in multiple cells. *Significant difference between MgTX and control \( (P \leq 0.05; \text{unpaired } t\text{-test}) \). Resolved Kv1.3 current was obtained by subtracting MgTX from control. B: ionic current measured in dissociated sympathetic neurons transfected with GFP-Kv1.3 in the absence (control; \( n = 3 \)) and presence of 1 nM MgTX \( (n = 3) \). Current traces for each condition represent the average of unpaired measurements made in multiple cells. *Significant difference between MgTX and control \( (P \leq 0.02; \text{unpaired } t\text{-test}) \). Resolved GFP-Kv1.3 current was obtained by subtracting MgTX from control. C: activation curves were plotted from the tail current of endogenous Kv1.3 and GFP-Kv1.3 and fit to a Boltzmann function. D: ionic current measured in dissociated sympathetic neurons in the absence (control; \( n = 10 \)) and presence of 100 nM DTX \( (n = 18; P > 0.05) \).
Kv1.3 current in nontransfected cells, indicating surface localization of the channel (Fig. 4 A). In nontransfected cells, surface expression of Kv1.3 was below the level of detection of our immunohistochemical analyses (Fig. 1 B). In neurons that were transfected with GFP-Kv1.3, surface expression was easily detectable (Fig. 3). In both nontransfected and transfected neurons, our immunohistochemical analyses detected a fraction of Kv1.3 localized to the Golgi apparatus (Figs. 2 and 3). Cellular localization is an important determinant of ion channel function (27). Our data thus suggest that Golgi localization or retention may be a determinant of surface expression and function of Kv1.3.

The primary preganglionic input to these neurons is ACh. It is well known that activation of mAChRs modulates KCNQ potassium channels in postganglionic sympathetic neurons and that this modulation affects neurotransmitter release (28). In addition, previous studies indicate that activation of mAChRs modulates the function of Kv1 channels (19). We show that activation of mAChRs suppresses Kv1.3 current in postganglionic sympathetic neurons (Fig. 6). These data demonstrate a novel effect of mAChR activation, a novel mechanism of Kv1.3 modulation, and suggest a new mechanism by which mAChR activation modulates membrane excitability.

In addition to activating mAChRs, release of ACh from preganglionic neurons activates nAChRs. Nicotinic AChR activation is the primary mechanism for generating action potentials and eliciting neurotransmitter release from postganglionic sympathetic neurons. We assessed the effects of Kv1.3 on nicotinic receptor-induced NE release. Our data indicate that inhibition of Kv1.3 enhanced NE release (Fig. 7). This suggests that Kv1.3 is acting to suppress membrane excitability and thereby inhibit neurotransmitter release. These findings are consistent with previous reports demonstrating that inhibition of Kv1 family channels enhances neurotransmitter release. The work of Jackson et al. (22) and Uhrenholt and Nedergaard (45) indicates that inhibition of Kv1 channels enhances NE release at sympathetic neurovascular junctions. Our findings that Kv1.3 channels are present in processes innervating arteries and that MgTX enhances NE release, suggest that these channels play a role in modulating sympathetic neurovascular transmission.

Our data clearly indicate that Kv1.3 channels influence the function of postganglionic sympathetic neurons derived from the SCG of the rat, a representative paravertebral sympathetic ganglion. The postganglionic sympathetic neurons in this ganglion innervate many targets, including blood vessels (30). Kv1.3 was expressed in all of the neurons that were studied, suggesting that Kv1.3 affects sympathetic regulation of all SCG targets. The studies of Dixon and McKinnon (5) indicate that Kv1.3 is also expressed in prevertebral ganglia, suggesting a general role for Kv1.3 in postganglionic sympathetic neuronal function. Our finding that Kv1.3 is expressed in the fibers innervating the tail artery (Fig. 1 C) strongly suggests that
Kv1.3 affects neurotransmitter release at sympathetic nervous junctions. This would suggest that Kv1.3 is a determinant of sympathetic control of blood flow and blood pressure.

**Perspectives and Significance**

Compelling evidence suggests that the sympathetic nervous system contributes to the development and/or maintenance of cardiovascular disease (2–4, 7, 9, 15, 23, 24, 29, 32, 40–42). Sympathetic activity is increased in hypertensive animals and humans, and sympathoinhibition decreases blood pressure (4, 15, 23, 24, 29, 40). Hypertension is also a complication of diabetes and obesity. Elevated sympathetic activity is thought to contribute to these forms of hypertension (2, 7). Sympathetic activity is increased in many patients with heart failure, and this increased activity contributes to the pathology of this disease (41, 42). The present studies suggest that Kv1.3 channels in postganglionic sympathetic neurons are determinants of sympathetic activity and therefore are potential therapeutic targets for the prevention and treatment of cardiovascular disease.

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