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J Neurophysiol 94:3849-3859, 2005. doi:10.1152/jn.00573.2005

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Submitted 1 June 2005; accepted in final form 22 August 2005

Belugin, Sergei and Steve Mifflin. Transient voltage-dependent potassium currents are reduced in NTS neurons isolated from renal wrap hypertensive rats. *J Neurophysiol* 94: 3849–3859, 2005; doi:10.1152/jn.00573.2005. Whole cell patch-clamp measurements were made in neurons enzymatically dispersed from the nucleus of the solitary tract (NTS) to determine if alterations occur in voltage-dependent potassium channels from rats made hypertensive (HT) by unilateral nephrectomy/renal wrap for 4 wk. Some rats had the fluorescent tracer DiA applied to the aortic nerve before the experiment to identify NTS neurons receiving monosynaptic baroreceptor afferent inputs. Mean arterial pressure (MAP) was greater in 4-wk HT (165 ± 5 mmHg, $n = 26$, $P < 0.001$) rats compared with normotensive (NT) rats (109 ± 3 mmHg measured in 10 of 69 rats). Transient outward currents (TOCs) were observed in 67–82% of NTS neurons from NT and HT rats. At activation voltages from -10 to $+10$ mV, TOCs were significantly less in HT neurons compared with those observed in NT neurons ($P < 0.001$). There were no differences in the voltage-dependent activation kinetics, the voltage dependence of steady-state inactivation, and the rise and decay time constants of the TOCs comparing neurons isolated from NT and HT rats. The 4-aminopyridine–sensitive component of the TOC was significantly less in neurons from HT compared with NT rats ($P < 0.001$), whereas steady-state outward currents, whether or not sensitive to 4-aminopyridine or tetraethylammonium, were not different. Delayed excitation, studied under current clamp, was observed in 60–80% of NTS neurons from NT and HT rats and was not different comparing neurons from NT and HT rats. However, examination of the subset of NTS neurons exhibiting somatic DiA fluorescence revealed that DiA-labeled neurons from HT rats had a significantly shorter duration delayed excitation ($n = 8$ cells, $P = 0.022$) than DiA-labeled neurons from NT rats ($n = 7$ cells). Neurons with delayed excitation from HT rats had a significantly broader first action potential (AP) and a slower maximal downstroke velocity of repolarization compared with NT neurons with delayed excitation ($P = 0.016$ and $P = 0.014$, respectively). The number of APs in the first 200 ms of a sustained depolarization was greater in HT than NT neurons ($P = 0.012$). These results suggest that HT of 4-wk duration reduces TOCs in NTS neurons, and this contributes to reduced delayed excitation and increased AP responses to depolarizing inputs. Such changes could alter baroreflex function in hypertension.

INTRODUCTION

Neurons within the nucleus of the solitary tract (NTS) integrate a variety of peripheral afferent inputs from visceral mechanoreceptors and chemoreceptors. Previous studies using microinjection and electrophysiological techniques have shown alterations in NTS neuronal responses to activation of GABAergic receptors in chronic hypertension (Mei et al. 2003; Tolstykh et al. 2003; Tsukamoto and Sved 1993; Yin and Sved 1996) and chronic hypoxia (Tolstykh et al. 2004). These changes are likely to contribute to altered reflex regulation of cardiorespiratory function in such chronic conditions.

Despite their importance as determinants of neuronal input–output relationships, surprisingly little attention has been directed toward possible alterations in voltage-gated potassium conductances in NTS neurons after chronic changes in physiological state. Delayed excitation is defined as a delay in the occurrence of an action potential (AP) response to depolarization after hyperpolarization and is primarily attributed to TOCs. Delayed excitation has been reported to be reduced in NTS neurons in the spontaneously hypertensive rat (Sundaram et al. 1997).

NTS neurons exhibit most major voltage-dependent potassium conductances (Bailey et al. 2002; Moak and Kunze 1993; Paton et al. 1993). NTS neurons receiving afferent inputs from the arterial baroreceptors express considerable voltage-dependent activation of transient outward potassium conductances (TOCs), and as a result, delayed excitation is an essential feature of most of these neurons (Bailey et al. 2002; Paton et al. 1993; Sundaram et al. 1997). Functionally, microinjection into the NTS of 4-aminopyridine (4-AP), an antagonist of TOCs and delayed excitation, attenuates baroreflex regulation of heart rate (Butcher and Paton 1998).

The goal of this study was to examine TOCs under voltage clamp and delayed excitation under current clamp to provide insights into possible changes in voltage-gated potassium channel function in NTS neurons in a nongenetic model of hypertension. If such changes occur in NTS neurons receiving baroreceptor inputs, they could contribute to alterations in cardiovascular regulation in hypertension.

METHODS

General

Experiments were performed on 95 adult, male Sprague-Dawley rats (375–500 g; Charles River Laboratories). Rats were housed two per cage in a fully accredited (Association for Assessment and Accreditation of Laboratory Animal Care and the United States Department of Agriculture) laboratory animal room with free access to food and water. All rats were given ≥1 wk to acclimate before being used for any procedures. The Institutional Animal Care and Use Committee approved all experimental protocols.

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Chronic hypertensive model

Rats were anesthetized with medetomidine (0.5 mg/kg, ip; Pfizer) and ketamine (75 mg/kg, ip; Fort Dodge Laboratory); under aseptic conditions, hypertension was induced using a figure-eight Grollman renal wrap and contralateral nephrectomy (Grollman A 1944). Normotensive (NT) rats were similarly anesthetized and received a unilateral nephrectomy but no wrap of the contralateral kidney (sham) or no surgical procedures before the day of the experiment. Because the neuronal responses of both groups of NT rats were identical, they were grouped for analysis. Anesthesia was terminated by atipamezole (1 mg/kg, ip; Pfizer) at the conclusion of the surgical procedures. Postoperative analgesics (Children's Tylenol, McNeil-PPC, po) were provided for the first 2–5 days after surgery. In three NT and four rats hypertensive (HT) for 4 wk, a second surgery was performed 2 wk after the first under the same anesthetic regimen and the anterograde fluorescent tracer DiA (Molecular Probes) was applied to the aortic nerve to visualize putative baroreceptor synaptic terminals and neurons receiving these synaptic contacts as previously described (Mendelowitz et al. 1992).

Experiments were performed 28–35 days (4-wk HT, $n = 26$) after renal wrap or sham surgery. Two days before the experiment, an arterial catheter was placed in the femoral artery while the animal was under medetomine/ketamine anesthesia (0.5 mg/kg, ip, and 75 mg/kg, ip, respectively). After a 2-day recovery period, blood pressure was measured in the conscious, freely moving rat by connecting the arterial catheter to a pressure transducer (Kobe) and visualized using MacLab or Cambridge Electronics Design A/D converters. Blood pressure was measured for 3 h, and measurements during the last hour were used as an index of mean arterial pressure (MAP). MAP was measured in every HT rat to ensure the rat was indeed HT. MAP was measured in only 10 of 69 NT rats, because our previous studies (Mei et al. 2003; Tolstykh et al. 2003) have never found a sham-operated or surgically naive rat that was HT. The properties of cells obtained from NT rats in which MAP was measured were no different from those of cells in rats in which MAP was not measured; therefore the data were pooled for analysis.

Dispersion of caudal NTS neurons

On the day of the experiment, the rat was anesthetized with isoflurane, and the brain stem was rapidly removed and placed in ice cold Krebs buffer. The brain stem was cut into 440-μm-thick transverse sections centered on calamus using a vibratome (Warner Instruments). The sections were incubated in PIPES buffer with trypsin (Sigma, type XI, 3–5 mg/10 ml) for 55 min at 34°C. After the enzyme treatment, slices were rinsed three times with incubation solution and were maintained at room temperature (22–25°C) in continuously oxygenated PIPES buffer. Brain stem slices were placed on a glass slide, and the NTS region was excised using a scalpel blade. The excised NTS region was gently triturated in a DMEM buffer using a series of fire-polished pipettes. After trituration, an aliquot of the DMEM/neuron suspension was placed in the perfusion bath and allowed to settle for 5 min, after which superfusion of the cells was begun with a solution consisting of (in mM) 140 NaCl, 33 D-glucose, 10 HEPES, 3 KCl, 2 $CaCl_2$, and 1.2 $MgCl_2$ (pH 7.4 with NaOH; osmolality 305–320 mOsm). All neurons were studied within 6 h after isolation.

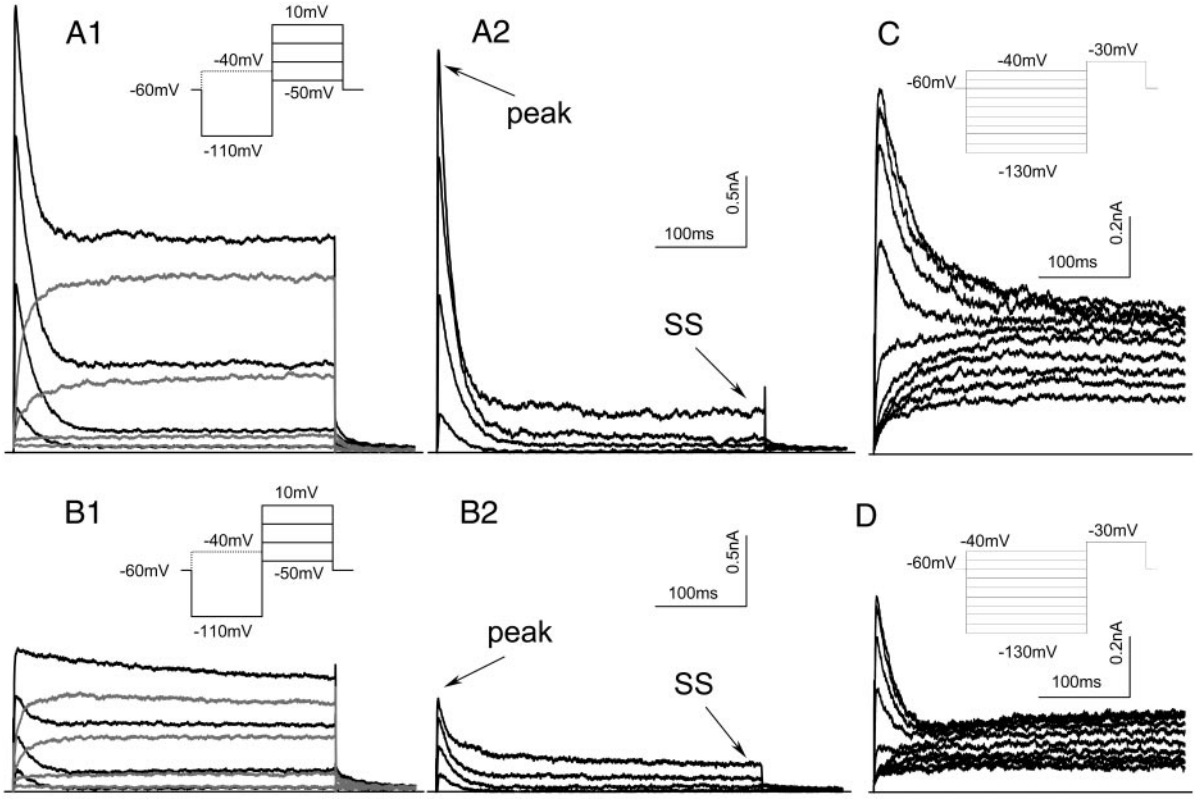

FIG. 1. Voltage-dependent activation (*A* and *B*) and steady-state inactivation (*C* and *D*) of outward currents in nucleus of the solitary tract (NTS) neurons from normotensive (NT; *A* and *C*) and hypertensive (HT; *B* and *D*) rats. *Insets*: voltage protocols used. In *A* (NT) and *B* (HT), neurons were held at −60 mV, and a hyperpolarizing command prepulse of either −110 (black line) or −40 mV (gray line) for 400 ms was followed by step-depolarizing commands to test potentials from −70 to 70 mV in 10-mV increments (for clarity, only 4 representative current responses are displayed). Responses obtained after a prepulse of −40 mV were subtracted from responses obtained after a prepulse of −110 mV to isolate the transient outward current (TOC; *A2* and *B2*), which consisted of both peak and steady-state (SS) components. In *C* (NT) and *D* (HT), steady-state voltage-dependent inactivation was measured as decay in TOC during a step to −30 mV after 1,000-ms preholding potentials of −130 to −40 mV.

Electrophysiology

Whole cell patch-clamp recordings were obtained from acutely dissociated NTS neurons with electrodes fabricated from quartz glass capillary tubing (1.0 mm OD, 0.70 mm ID) using a laser based P-2000 puller (Sutter Instrument). Electrodes were filled with a solution of (in mM) 140 KCl, 2 $MgCl_2$, 10 EGTA, 0.1 GTP, 4 ATP, and 10 HEPES (pH 7.2 with KOH; osmolality 280–300 mOsm) and had resistances of 3–8 MΩ when tested in bath solution. Voltage- and current-clamp experiments were performed using an Axopatch-200B integrating amplifier (Axon Instruments, Foster City, CA) and pCLAMP acquisition software (version 8.1, Axon Instruments) on a Pentium-class PC. Gigaseals (>1 GΩ) were formed, and whole cell recording configuration was obtained by suction. To maximize potassium currents and reduce sodium and calcium currents in voltage-clamp experiments, external solutions were Ca^{2+} free and contained TTX (0.5 μM). In current-clamp experiments, the external solution contained 2 mM $CaCl_2$, and TTX was excluded.

At rest in voltage- or current-clamp protocols, neurons were clamped at −60 mV or 0 pA, respectively. In voltage-clamp experiments, currents were expressed as current density (pA/pF) to normalize for cells of different sizes. Currents were low-pass filtered at 1 or 5 kHz and sampled at 5 or 20 kHz. Cells were included if leak current was <200 pA. Series resistance was <15 MΩ and compensated between 40 and 70%. Before each recording, a membrane test was performed to ensure that access resistance did not change over time or during different experimental conditions. Voltage step protocols for analysis of the activation kinetics of transient and steady-state outward currents consisted of 500-ms steps from −70 to +70 mV in 10- or 20-mV increments applied after a 400-ms conditioning step of either −110 or −40 mV (Fig. 1*A*). Steady-state voltage-dependent inactivation of transient outward currents (TOCs) was estimated using conditioning steps of 1,000 ms ranging from −130 to −40 mV in 10-mV increments followed by a test pulse of 500 ms to −30 mV (Fig. 1*C*). Reversal potentials were estimated by evaluation of the tail components of the outward currents generated by 50-ms pulses between −110 and −40 mV after a test pulse to 0 mV for 125 ms from a holding potential of −60 mV in 3 mM KCl and 20 mM KCl external solutions.

Two stimulus protocols were used in current-clamp experiments to evoke AP discharge: *1*) depolarizing current pulses of 40 pA (1,000 ms) from the resting membrane potential (holding current 0 pA) and *2*) depolarizing current pulses of 40 pA (1,000 ms) from a hyperpolarized membrane potential (holding current −100 pA). In current clamp, all data were collected in I-Clamp Fast mode, low-pass filtered to 5 kHz, and digitized at the rate of 20 kHz.

Solutions and drugs

The following solutions were made in the extracellular bath solution: 0.5 μM TTX (Sigma, St. Louis, MO), 5 mM 4-AP (Sigma), and 10 mM tetraethylammonium (TEA; Sigma). A two-barrel pipette array (500 μm ID) controlled by a fast-step perfusion apparatus (Warner Instruments) was used to apply drugs to isolated cells by

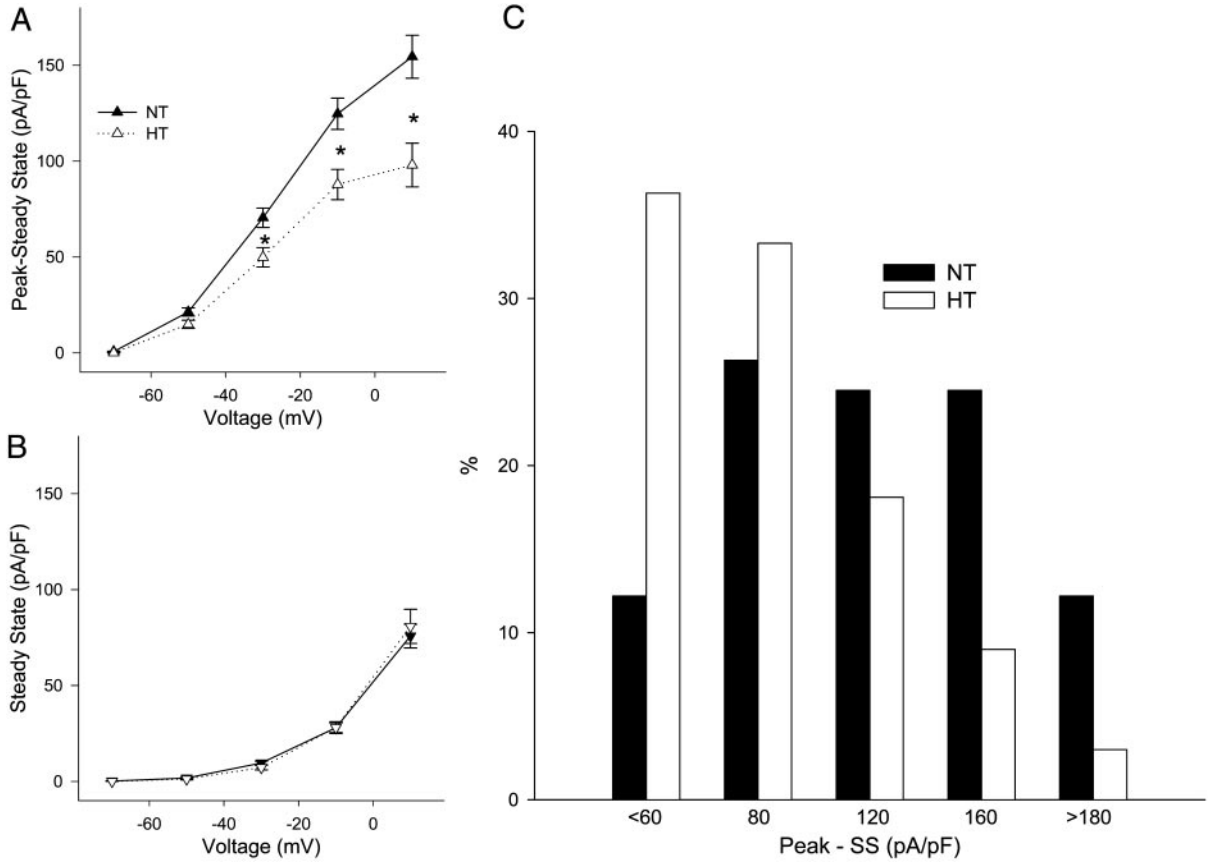

FIG. 2. Mean amplitude of TOCs (measured as the peak − steady-state values) (*A*) and steady-state outward currents (*B*) in NTS neurons isolated from NT and HT rats. Voltage step protocols were defined and shown in Fig. 1. *A*: 4-wk HT neurons (dotted line) had a significantly lower TOC compared with NT neurons (solid line; $P < 0.001$, 2-way ANOVA). *B*: steady-state outward currents were similar in 4-wk HT (dotted line) and NT (solid line) neurons. *C*: distribution of TOC amplitudes in HT (white bars) and NT (black bars) NTS neurons. TOCs measured at depolarizing pulse −10 mV and all cells with TOCs ≥ 30 pA/pF were taken into account. Population of neurons from 4-wk HT rats was shifted toward smaller TOCs than neurons from NT rats, although there is some overlap (between 30 and 60 pA/pF, $n = 7$ NT and 12 HT; between 60 and 100 pA/pF, $n = 15$ NT and 11 HT; between 100 and 140 pA/pF, $n = 14$ NT and 6 HT; between 140 and 180 pA/pF, $n = 14$ NT and 3 HT; between 180 and 220 pA/pF, $n = 7$ NT and 1 HT).

gravity flow. Each pipette was attached by tubing to valves connecting each pipette to the solutions. The first pipette was positioned adjacent and as close as possible to the target cell. A steady stream of TTX-containing bath solution abolished AP discharge and protected the neuron from leakage from the second downstream drug-containing pipette. To apply TEA or 4-AP, a digital signal was sent to the Warner Perfusion Fast-Step by means of a Clampex recording protocol, and the pipette assembly was rapidly moved so that the TTX-containing pipette was upstream to the cell and the TEA/4-AP–ejecting pipette was adjacent to the cell. After a user-defined interval, the pipette assembly was returned to the starting position.

Data analysis

The junction potential between the external and electrode solutions was approximately −4 mV, and membrane potentials were not corrected with this value. On-line leak subtraction was not employed. Off-line analyses were done using Clampfit Software (Axon) and Sigma Plot 8.1.

The conductance of the outward current (G) was calculated as $G = I\text{peak}/(V\text{m} - E\text{k})$. The relationship between normalized conductance and membrane potential (Vm) were fitted by the Boltzmann equation: $G/G_{\max} = \{1 + \exp[-(V\text{m} - V\text{h})/V\text{c}]\}^{-1}$, where $G_{\max}$ is the maximal membrane conductance, Vh is the voltage at half-maximal conductance, and Vc is the slope factor. Voltage-dependent inactivation of the TOC was fitted to the following equation: $(I - I\text{o})/I_{\max} = \{1 + \exp[(V\text{m} - V\text{h})/V\text{c}]\}^{-1}$, where I is the current measured in the steady-state voltage-dependent inactivation protocol and Io is the current evoked in the absence of a hyperpolarizing prepulse.

Current-clamp recordings were analyzed off-line to measure resting membrane potential, input resistance, the repetitive discharge characteristic of the neuron (frequency and interspike intervals of APs during the 1st 200 ms), duration of delayed excitation and the parameters of the first generated AP [amplitude (mV), half-width (ms), maximum upstroke velocity (mV/ms), maximum downstroke velocity (mV/ms), duration and amplitude of the afterhyperpolarization (ms and mV, respectively)]. Input resistance was calculated by measuring the average membrane potential responses to constant-amplitude current pulses (100 pA; 500 ms). All data described in this study are expressed as means ± SE. *I-V* curves were compared using two-way ANOVA with Fisher least significant difference (LSD) post hoc testing. Student's two tailed *t*-test was used for between-group comparisons. Differences in the relative distributions were tested using χ^2. Significance was accepted if $P < 0.05$.

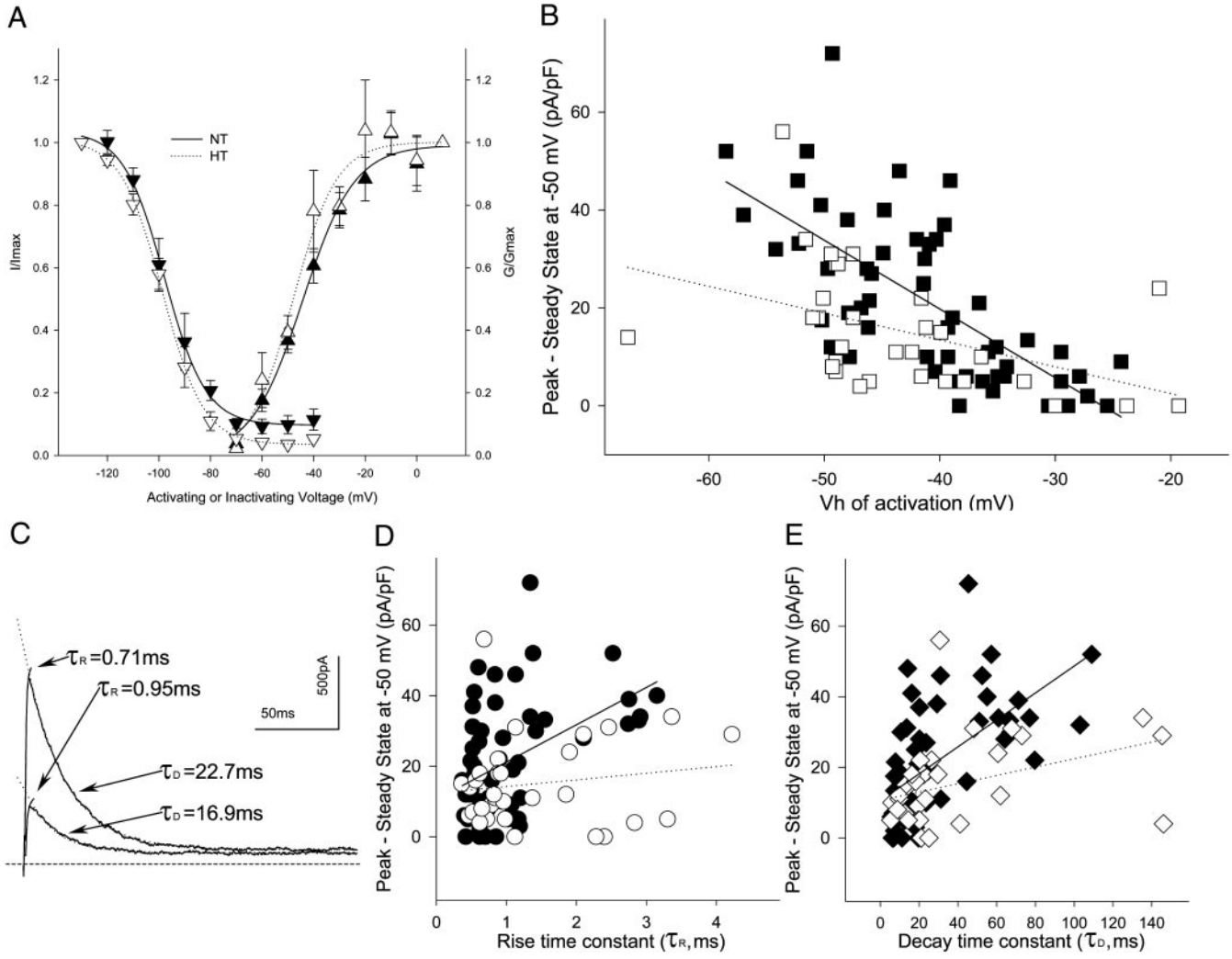

FIG. 3. *A*: activation and inactivation of TOCs of NT and HT neurons from NTS. Voltage protocols are shown in Fig. 1. Average normalized TOC amplitudes (peak − steady state) ± SE are plotted against activation voltage or conditioning voltage. These voltage-dependent activation and inactivation characteristics of TOCs are similar in NT and HT neurons from NTS and resemble those of A-type, 4-AP–sensitive potassium currents. *B, D*, and *E*: relationships between amplitude of TOCs (at −50 mV) and half-voltage of the steady-state activation (Vh), rise time constant (τR), and decay time constant (τD) of the TOCs, respectively, from individual neurons in NT (filled symbols) and 4-wk HT (open symbols). *C*: τR and τD from an NT (solid line) and 4-wk HT (gray line) neuron.

RESULTS

General

These experiments utsed 69 NT rats (MAP = 109 ± 3 mmHg measured in 10 rats) and 26 rats studied after 4-wk HT (MAP = 165 ± 5 mmHg). MAP was significantly elevated in 4-wk HT compared with NT rats ($P < 0.001$).

Dispersed NTS neurons were studied using either a voltage-clamp protocol (bath contained TTX and was Ca^{2+} free: 85 neurons in NT and 47 neurons in HT) or a current-clamp/voltage-clamp protocol (bath contained Ca^{2+} and was TTX free: 83 neurons in NT and 26 neurons in 4-wk HT). Cells with somatic DiA fluorescence were studied in the current-clamp/voltage-clamp protocol (7 neurons in NT, 8 neurons in 4-wk HT).

Voltage-clamp experiments (bath contained TTX and was Ca^{2+} free)

In NTS neurons isolated from both NT and HT rats, depolarization from a conditioning potential of −110 mV elicited large, voltage-dependent outward currents that decayed over time (Fig. 1, *A1* and *A2*, black lines). When depolarized from a relatively depolarized conditioning potential of −40 mV, these TOCs were absent and only non- or slowly inactivating delayed outward currents were observed (Fig. 1, *A1* and *B1*, gray lines). The amplitude of the TOCs was calculated by subtracting the steady-state level from the peak (Fig. 1, *A2* and *B2*). TOCs of >30pA/pF at −10 mV were observed in 57 of 85 neurons from NT rats (67%) and in 33 of 47 neurons from 4-wk HT rats (70%).

Membrane capacitance was not different in neurons with TOCs studied in NT (5.7 ± 0.2 pF, $n = 57$) or 4-wk HT (6.2 ± 0.5 pF, $n = 33$, $P = 0.24$ vs. NT). The reversal potential of the outward currents shifted from −84 ± 7 mV to −54 ± 9 mV when the external solution was changed from 3 mM KCl to 20 mM KCl ($n = 3$ neurons in NT). At 3 mM KCl, the measured potassium equilibrium potential was −83 ± 2 mV in NT ($n = 19$) and −81 ± 2 mV in 4-wk HT ($n = 15$; $P = 0.42$).

The amplitude of TOCs differed significantly between NTS neurons from NT and 4-wk HT rats. At activation voltages

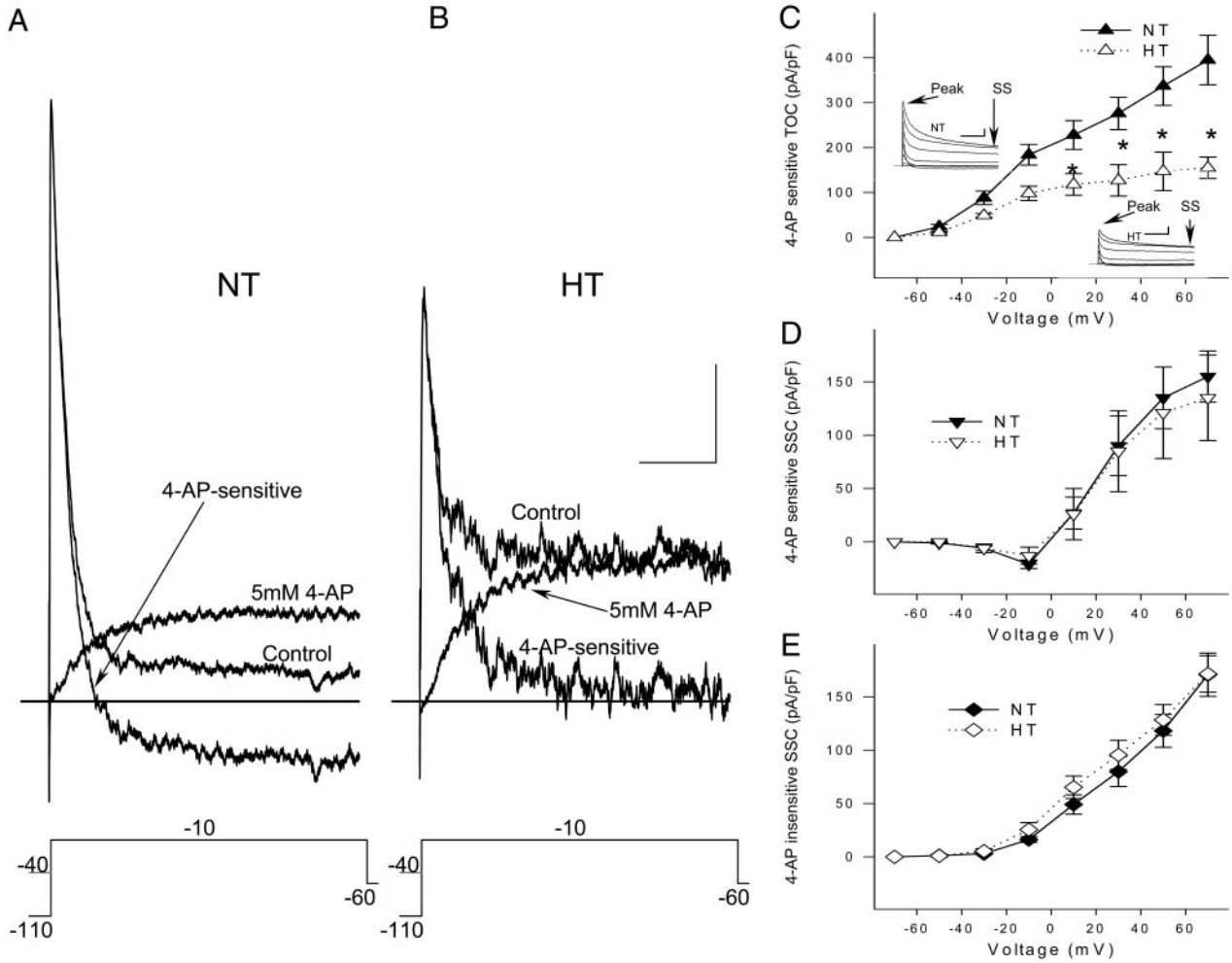

FIG. 4. Sensitivity of outward currents in NT ($n = 11$) and 4-wk HT ($n = 8$) NTS neurons to 5 mM 4-AP. In *A* (NT) and *B* (HT), control and 5 mM 4-aminopyridine (4-AP) traces are the result of subtraction of currents generated by a command voltage step to −10 mV from a preholding pulse of either −110 or −40 mV applied before and after superfusion of cells with 5 mM 4-AP. Trace marked 4-AP sensitive is the result of subtraction of the 5 mM 4-AP trace from the control trace. Bars, horizontal −100 ms, vertical −50 pA/pF. *Insets* in *C* (NT and HT) were obtained by subtracting the control and 5 mM 4-AP traces to yield the net 4-AP–sensitive current. In *C*, 4-AP–sensitive TOCs were significantly reduced in HT neurons compared with NT neurons at activation voltages from +10 to +70 mV ($P < 0.001$; 2-way ANOVA). 4-AP–sensitive steady-state outward currents (SSCs) (*D*) and 4-AP–insensitive SSCs (*E*) were similar in NT and HT neurons. These results suggest that the TOC observed in HT and NT neurons of NTS is an A-type potassium current that is decreased in HT neurons.

from −30 to +10 mV, TOCs were significantly less in 4-wk HT neurons compared with those observed in NT neurons (Fig. 2*A*; $P < 0.001$). As a percentage of the total population, more NTS neurons from 4-wk HT rats had smaller peak TOCs than did NTS neurons from NT rats ($\chi^2 = 11.14107$; $P = 0.025$; Fig. 2*C*). The steady-state component of the outward current was not different between neurons from NT and 4-wk HT rats (Fig. 2*B*).

From hyperpolarized holding potentials (−110 mV), depolarization activated TOCs with similar voltage-dependent activation kinetics in both NT (Vh = −41.4 ± 1.1 mV, Vc = 8.6 ± 0.5; $n = 55$) and 4-wk HT (Vh = −43.4 ± 1.8 mV, Vc = 8.7 ± 0.8; $n = 31$) NTS neurons (Fig. 3*A*). The voltage dependence of steady-state inactivation was no different between neurons from NT and HT rats (Fig. 3*A*). In neurons from NT rats, the half-inactivation voltage (Vh) of the peak current was −96.3 ± 3.3 mV, and Vc was −6.1 ± 0.4 ($n = 7$). In 4-wk HT rats, Vh was −97.6 ± 2.6 mV, and Vc was −7.4 ± 0.6 ($n = 11$). The rise time constants of TOC activation were no different in 4-wk HT neurons (τR = 1.35 ± 0.17 ms; $n = 33$) compared with NT neurons (τR = 1.01 ± 0.09 ms; $n = 57$; Fig. 3*C*). The decay time constants were no different in 4-wk HT neurons (τD = 36.1 ± 6.7 ms; $n = 33$) compared with NT neurons (τD = 28.2 ± 3.4; $n = 57$).

Figure 3, *B*, *D*, and *E*, shows the relationships between the half-voltage of steady-state activation (Vh), monoexponential rise time constant of TOC activation (τR), and monoexponential decay time constant of TOC inactivation (τD), and the corresponding values for TOC amplitude at −50 mV. The slopes of the relationships between the amplitude of the TOC and the kinetic parameters (Vh, τR, τD) were significantly less in HT neurons compared with NT neurons (Fig. 3, *B*, *D* and *E*). The relationship between TOC amplitude and Vh was linear in neurons from NT (correlation coefficient = −0.69, $P < 0.0001$, $n = 54$) and HT (correlation coefficient = −0.44, $P = 0.012$, $n = 31$) rats. The slope of this relationship was less in HT (−0.54 ± 0.19 current density/10 mV) than in NT (−1.40 ± 0.20 current density/10 mV; $P = 0.007$). The

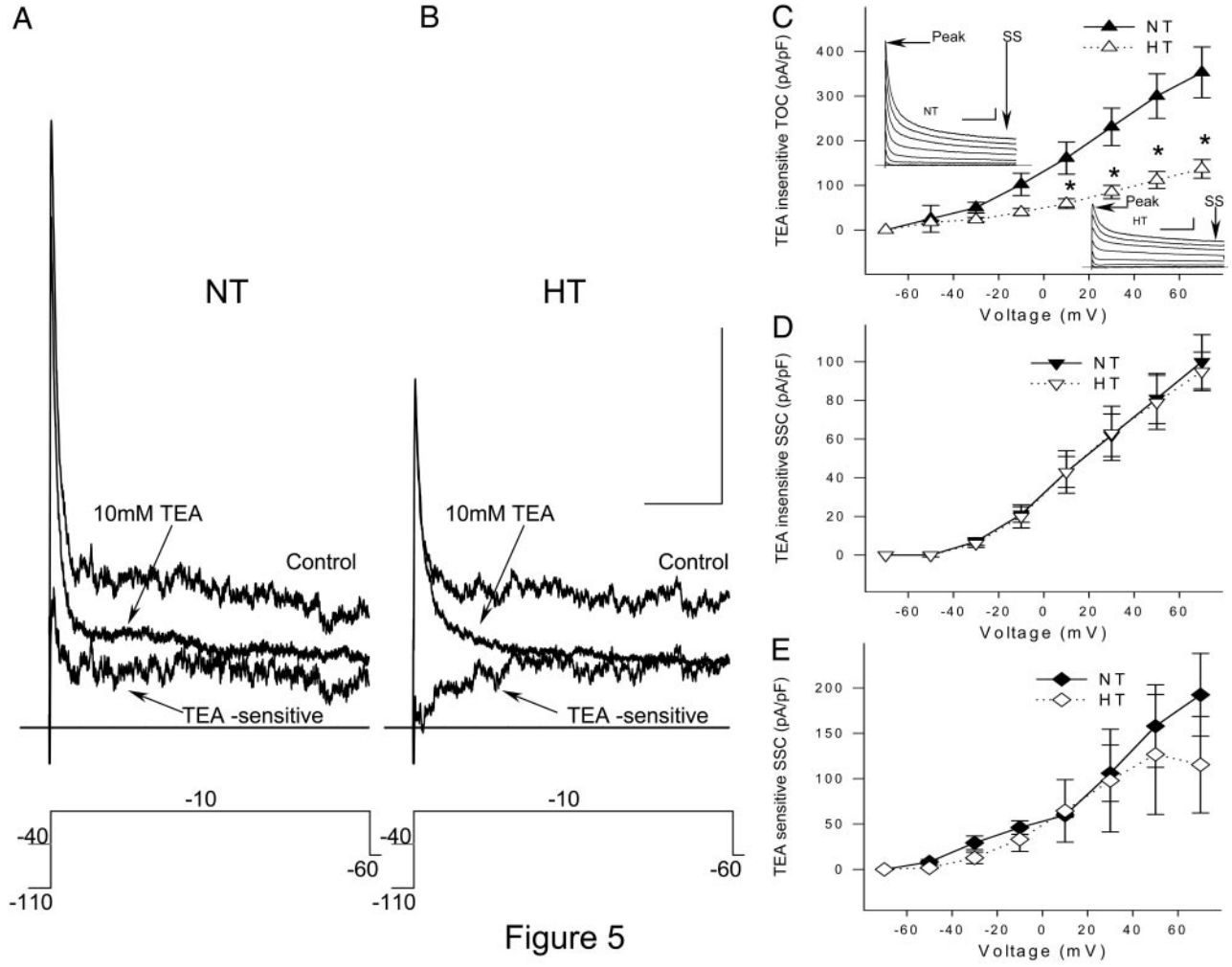

Figure 5

FIG. 5. Sensitivity of outward currents to 10 mM tetraethylammonium (TEA) in NT ($n = 8$) and 4-wk HT ($n = 7$) NTS neurons. In *A* (NT) and *B* (HT), control and TEA traces are the result of subtraction of currents generated by a command voltage step to −10 mV from a preholding pulse of either −100 or −40 mV applied before and after superfusion of cells with TEA. Trace marked TEA sensitive is the result of subtraction of the TEA trace from the control trace. Bars, horizontal −100 ms, vertical −50 pA/pF. *Insets* in *C* (NT and HT) were obtained by subtracting the control and TEA traces to yield the net TEA-sensitive current. In *A* (NT) and *B* (HT) TEA reduced the steady-state outward currents (SSCs) but did not affect TOCs. In *C*, TEA-insensitive TOCs were significantly reduced in HT neurons compared with NT neurons at activation voltages from +10 to +70 mV ($P < 0.001$; 2-way ANOVA). TEA-insensitive SSCs (*D*) and TEA-sensitive SSCs (*E*) were similar in NT and HT neurons.

relationship between TOC amplitude and τR was linear in NT (correlation coefficient = 0.46, $P < 0.0003$, $n = 57$) but not in HT (correlation coefficient = 0.15, $P = 0.39$, $n = 33$). The slope of this relationship was less in HT (1.88 ± 2.19 current density/10 ms) than in NT (10.58 ± 2.71 current density/10 ms; $P = 0.03$). The relationship between TOC amplitude and τD was linear in NT (correlation coefficient = 0.60, $P < 0.0001$, $n = 57$) and HT (correlation coefficient = 0.37, $P < 0.033$, $n = 33$). The slope of this relationship was less in HT (0.12 ± 0.05 current density/10 ms) than in NT (0.38 ± 0.07 current density/10 ms; $P < 0.008$).

Pharmacological characterization of outward currents in NTS neurons from NT and HT rats

Bath application of 5 mM 4-AP was used to block TOCs in NTS neurons from both NT and 4-wk HT rats (Fig. 4, *A* and *B*). The 4-AP–sensitive component of the TOC was significantly less in neurons from HT compared with NT rats (Fig. 4*C*; $P < 0.001$). Neither the steady-state 4-AP–sensitive (Fig. 4*D*) nor the steady-state 4-AP–insensitive (Fig. 4*E*) outward currents were different comparing neurons from NT and HT rats.

TEA (10 mM) suppressed the sustained outward currents in NTS neurons of NT and HT rats without altering the TOCs (Fig. 5, *A* and *B*). TEA-insensitive TOCs were significantly less in neurons from HT rats than in NT rats (Fig. 5*C*; $P < 0.001$). Neither the steady-state TEA-insensitive (Fig. 5D) nor the steady-state TEA-sensitive (Fig. 5*E*) outward currents were different comparing neurons from NT and HT rats.

Current-clamp experiments (bath contained no TTX and 2 mM $CaCl_2$)

To correlate AP discharge patterns with voltage-dependent outward currents, current-clamp and voltage-clamp recordings were obtained from the same NTS neurons isolated from NT and HT rats. There was no difference in the resting membrane potential or input resistance comparing neurons isolated from NT (−54 ± 1 mV, 879 ± 32 MΩ, $n = 52$) and 4-wk HT (−58 ± 4 mV, 981 ± 60 MΩ, $n = 20$) rats.

Examples of the AP discharge patterns in response to depolarizing step pulses under current-clamp conditions are shown in Fig. 6 for a NT neuron (Fig. 6*A1*) and a HT neuron (Fig. 6*B1*). From a holding potential of −100 pA, a depolarizing pulse to +40 pA evoked either a delay in the first AP generated or a long delay between the first and second APs generated. Both were considered delayed excitation if the duration of the delays were >30 ms.

Delayed excitation was observed in 52 of 83 NT neurons (63%) and in 20 of 26 4-wk HT neurons (77%). The duration of the delayed excitation was not different in the neurons from NT rats (445 ± 53 ms) compared with neurons from 4-wk HT rats (295 ± 83 ms; $P = 0.138$).

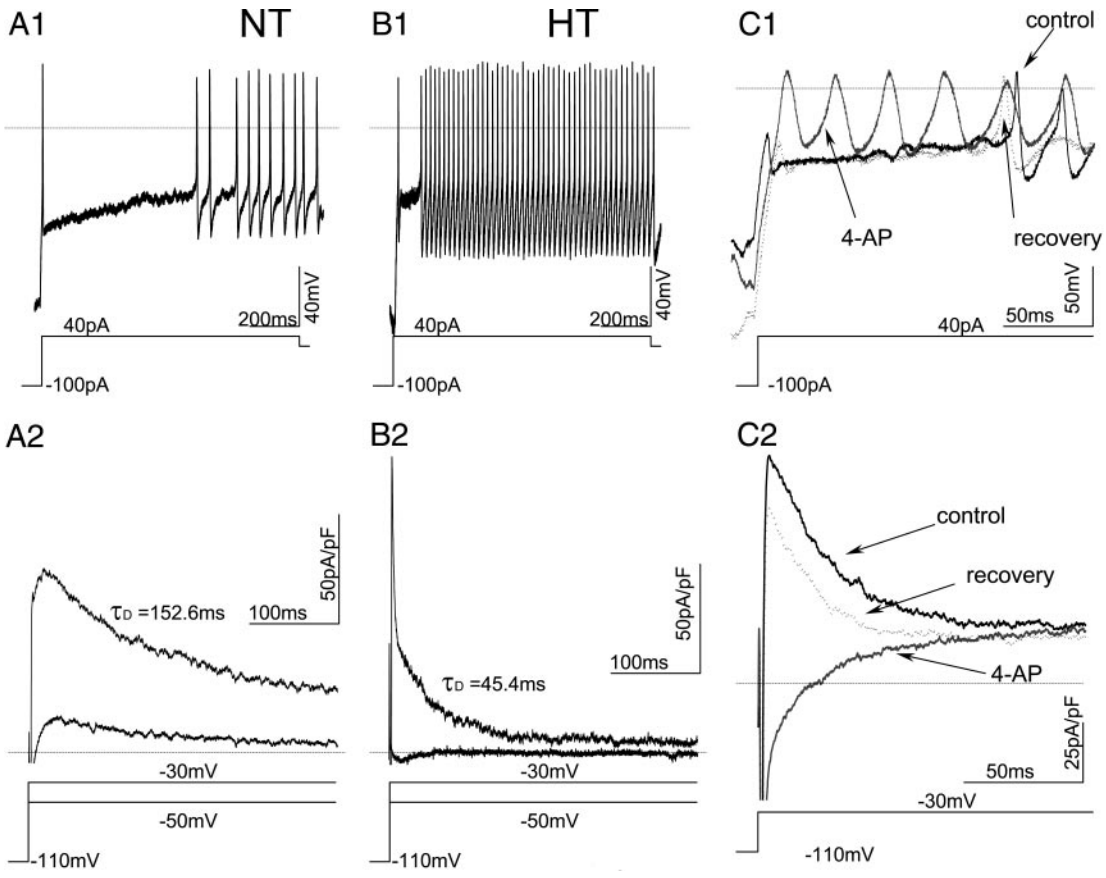

FIG. 6. Delayed excitation in NT (*A1*) and HT (*B1*) NTS neurons and outward currents in the same neurons (*A2* and *B2*). *C*: 4-AP effect on delayed excitation (*C1*) and TOC (*C2*) of NTS neuron. *C1*: application of 4-AP shortened the latency for the 1st action potential (AP) and prolonged duration of the AP. *C2*: application of 4-AP to the same neuron as in *C1* abolished the TOC. Dashed horizontal lines indicate 0 level of voltage or current.

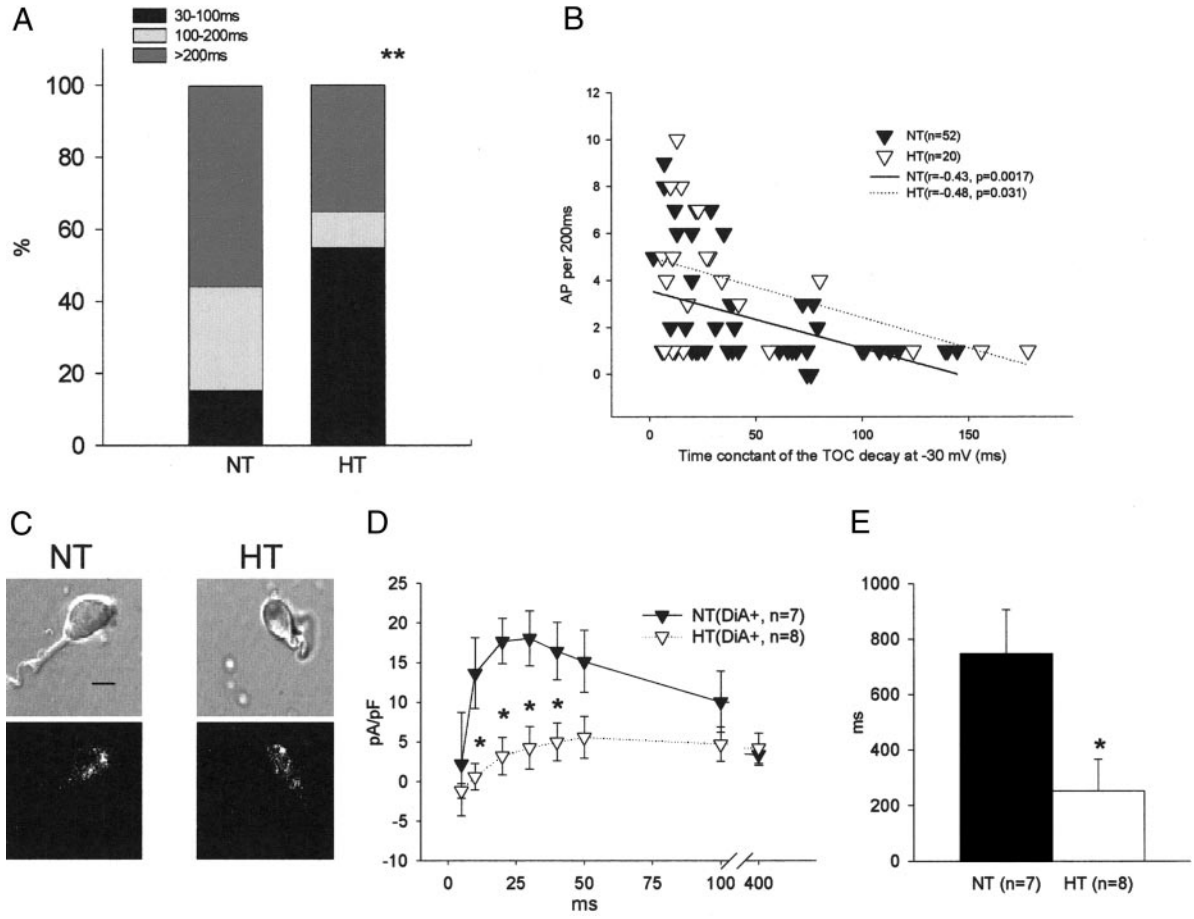

FIG. 7. *A*: distribution of the duration of delayed excitation in cells with a delayed excitation >30 ms. NT neurons ($n = 52$): 8 cells with delayed excitation between 30 and 100 ms, 15 between 100 and 200 ms, and 29 >200 ms. Four-week HT ($n = 20$): 11 cells with delayed excitation between 30 and 100 ms, 2 between 100 and 200 ms, and 7 >200 ms. χ^2 test for NT neurons and 4-wk HT neurons: $P = 0.002$. *B*: relationship between number of APs during 1st 200 ms of a depolarizing (+40 pA) pulse and time constant of decay of outward current at −30 mV. There is a negative correlation between number of APs and time constant for decay of the TOC in NT and HT neurons. *C*: NTS NT and HT neurons with DiA fluorescence from aortic depressor nerve–labeled terminals on outer edges of soma. Horizontal bar = 5 μm. *D*: outward currents evoked from a conditioning potential of −110 mV by a depolarizing voltage step to −50 mV (400 ms) were significantly smaller in DiA-labeled neurons from 4-wk HT compared with NT rats. *E*: delayed excitation was significantly shorter in DiA-labeled HT neurons compared with DiA-labeled NT neurons.

However, examination of the subset of NTS neurons exhibiting somatic DiA fluorescence and therefore a presumptive monosynaptic aortic nerve input (Fig. 7*C*) revealed that DiA-labeled neurons from NT rats had significantly larger TOCs than DiA-labeled neurons from 4-wk HT rats at a membrane potential of −50 mV, close to the threshold of TOCs (Fig. 7*D*). DiA-labeled neurons from 4-wk HT rats also exhibited a delayed excitation with significantly shorter duration (252 ± 115 ms, $n = 8$, $P = 0.022$) than DiA-labeled neurons from NT rats (748 ± 152 ms, $n = 7$; Fig. 7*E*).

The rate of recovery from inactivation was measured for the TOC by inactivating TOCs by a preholding potential of −30 mV for 300 ms and then stepping to −130 mV for increasing periods (10-ms increments before stepping to a test pulse of −30 mV; Fig. 8*A*). While studied in a small number of cells, there was no obvious difference between the time constant for recovery from inactivation comparing NT to HT neurons (Fig. 8*B*). In the NT group, the time constant for recovery from inactivation for two DiA-labeled cells was 7.8 and 29.3 ms, and in the HT group, time constant for recovery from inactivation for two DiA-labeled cells was 21.3 and 23.5 ms.

AP parameters in NTS neurons with delayed excitation

AP parameters were estimated in NT and HT neurons under conditions of minimal activation of TOCs (holding current of 0 pA followed by depolarizing pulse to +40 pA; Fig. 9*A*) and conditions of maximal activation of TOCs (holding current of −100 pA followed by depolarizing pulse to +40 pA; Fig. 9*B*). Under conditions of minimal activation of TOCs, there was no difference in any measured AP parameters comparing neurons from NT and HT rats (Fig. 9*A2*). Neurons isolated from 4-wk HT rats with delayed excitation had a significantly broader first AP (2.32 ± 0.61 ms, $n = 20$) and a slower maximal downstroke velocity of repolarization (60 ± 4 mV/ms, $n = 20$) in comparison to NT neurons with delayed excitation (1.34 ± 0.06 ms and 76 ± 3 mV/ms, $P = 0.016$ and $P = 0.014$, respectively, $n = 51$; Fig. 9*B2*). The number of APs in the first 200 ms of a sustained depolarization was greater in HT (3.9 ± 0.6 APs, $n = 20$) than NT neurons (2.3 ± 0.3 APs, $n = 52$, $P = 0.012$).

DISCUSSION

Previous studies have shown alterations in ligand-gated conductances in NTS neurons after chronic changes in physi-

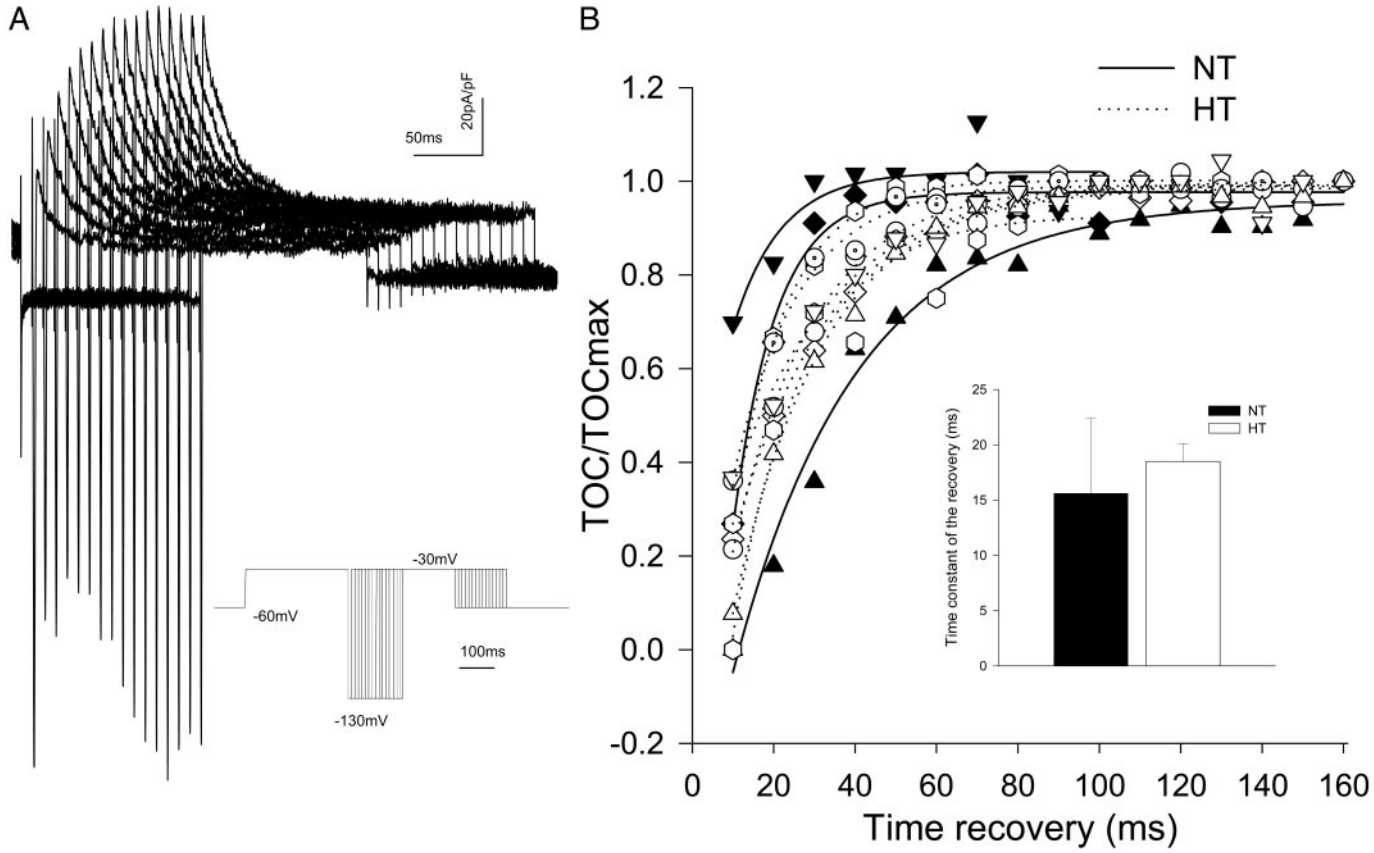

FIG. 8. *A*: recovery from inactivation was examined by inactivating TOCs by a preholding potential of −30 mV for 300 ms and stepping to −130 mV for increasing periods (10-ms increments before stepping to a test pulse of −30 mV). This voltage-clamp protocol was performed on cells in medium with 2 mM $CaCl_2$ and no TTX present. *B*: plot of TOC/TOC_{max} as a function of prepulse duration at −130 mV. Data were fit with as a single exponential $TOC_{max}[1 - \exp(-t/\tau)]$ + TOC; solid and dotted lines }, and the bar histogram gives mean values of recovery time constant in NT ($n = 3$) and HT neurons ($n = 7$). Data from NT neurons is represented by filled symbols and by open symbols for HT. Results from DiA-labeled cells are marked by filled (NT) or open (HT) triangles.

ological state such as hypertension (Mei et al. 2003; Tolstykh et al. 2003; Tsukamoto and Sved 1993; Yin and Sved 1996) and hypoxia (Tolstykh et al. 2004). These results extend the idea that NTS neurons are capable of adapting to a change in physiological state and indicate that alterations in the responses to activation of voltage-gated potassium channels occur after prolonged changes in physiological state as well.

The presence of neurons in NTS with transient outward potassium currents that are sensitive to 4-AP and insensitive to TEA has been shown by a number of studies (Bailey et al. 2002; Moak and Kunze 1993; Paton et al. 1993). NTS neurons with transient outward potassium currents usually exhibit delayed excitation (Bailey et al. 2002; Paton et al. 1993), and delayed excitation in NTS neurons is blocked by 4-AP (Paton et al. 1993). NTS neurons with prominent delayed excitation are most likely second-order neurons in the arterial baroreflex receiving C-fiber afferent inputs (Bailey et al. 2002). While it is likely that the delay to the first AP is only partly mediated by TOCs (Hattori 2003; Liss 2001; Sundaram et al. 1997), our analysis revealed no differences in NT and HT neurons in delayed and steady-state TEA-sensitive K-currents in Ca^{2+}-containing or Ca^{2+}-free solutions.

While the contribution of TOCs to the responses of NTS neurons to depolarization has been repeatedly shown, the significance of TOCs in the regulation of neuro-humoral status has been difficult to discern. Microinjection of 4-AP into the NTS resulted in a fall in MAP, consistent with enhanced baroreflex inhibition of sympathetic outflow; however, baroreflex-mediated bradycardia was reduced, suggesting inhibition of baroreflex function (Butcher and Paton 1998). The authors proposed a preferential localization of TOCs on GABAergic neurons within the NTS; however, this seems unlikely given the observation that the majority of NTS neurons exhibit 4-AP–sensitive TOCs.

These results indicate that TOCs were significantly less in NTS neurons from 4-wk HT rats compared with neurons from NT rats. Consistent with this observation, 4-AP–sensitive TOCs were less in the HT neurons than in NT neurons. Current-clamp recordings indicate that the reduction in TOCs might have functional significance in baroreceptor afferent integration as NTS neurons from HT rats exhibit reduced delayed excitation and increased AP discharge in response to depolarization.

A previous study reported a reduced duration of delayed excitation in NTS neurons from spontaneously hypertensive rats (Sundaram et al. 1997); therefore the changes reported here may be a general consequence of hypertension regardless of the underlying etiology of the hypertension. The A-type K channel (I_A) has also been reported to be altered in sympathetic, superior cervical ganglia in spontaneously hypertensive rat (Robertson and Schofield 1999); however, the mechanisms appear more complicated than reported for NTS neurons in this report. In the spontaneously hypertensive rat sympathetic ganglia, I_A amplitude and current density are unchanged; however,

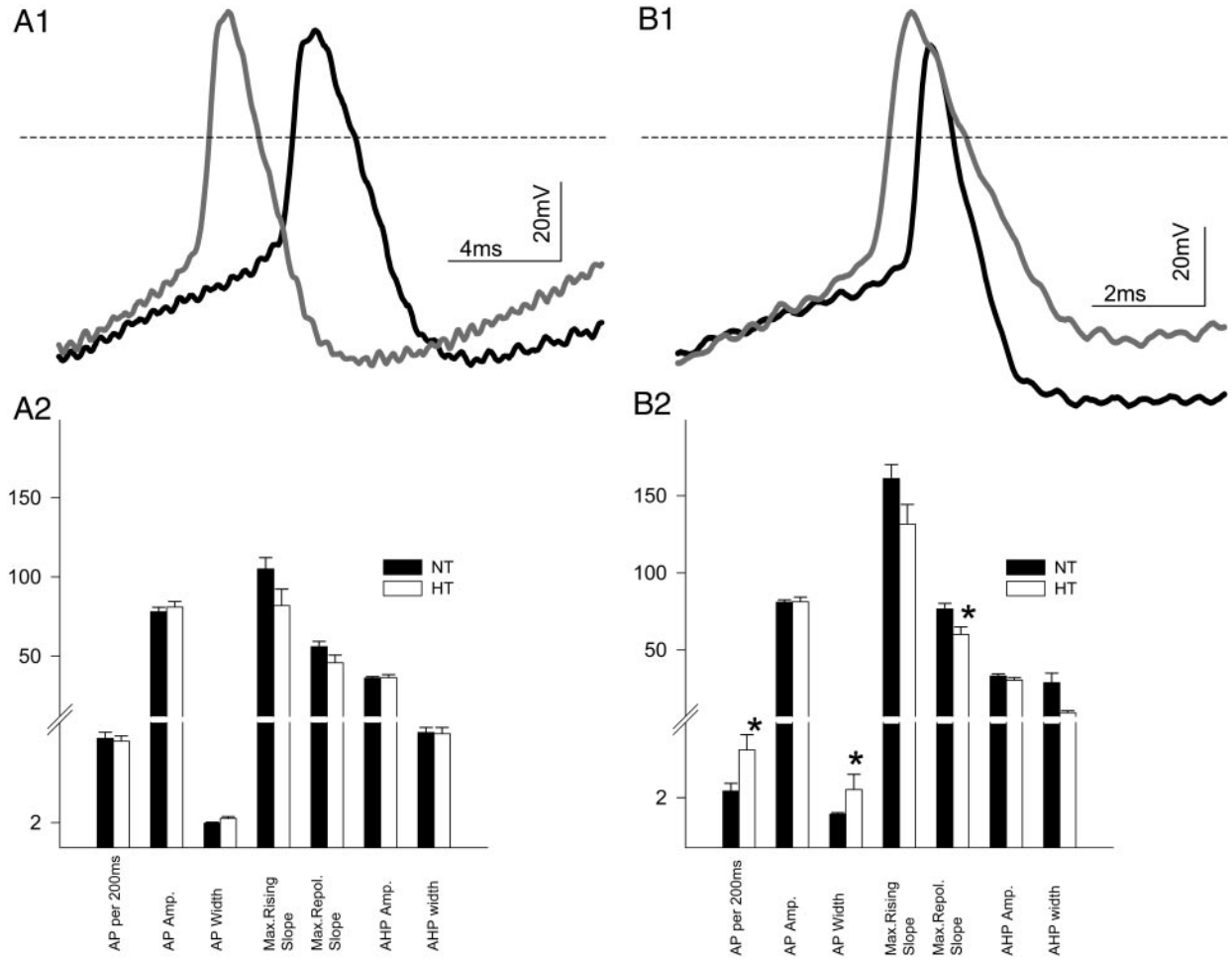

FIG. 9. AP parameters in NTS neurons from NT and 4-wk HT rats. *A1* and *A2*: if cells are depolarized by a +40-pA current injection from a holding current of 0 pA (resting membrane potential), AP parameters are the same in NT and HT neurons. *B1* and *B2*: if cells are depolarized by a +40-pA current injection from a holding current of −100 pA, AP in HT rats is significantly shorter in duration and exhibits a faster speed of repolarization compared with NT. *A1* and *B1*: examples of APs in neurons from NT (black lines) and HT (gray lines) rats. *A2* and *B2*: histograms comparing AP parameters in NT ($n = 51$) and HT ($n = 21$) neurons. AP per 200 ms, number of APs in the 1st 200 ms after depolarization; AP Amp., AP amplitude; AP width, duration of AP; Max Rising Slope, slope of ascending limb of AP; Max Repol. Slope, slope of descending limb of AP; AHP amp., afterhyperpolarization amplitude; AHP width, afterhyperpolarization duration.

steady-state inactivation is shifted to a more hyperpolarized level. Reductions in potassium conductances occur in vascular smooth muscle during hypertension (Cox and Rusch 2002) and have been proposed to contribute to the elevated vascular tone. Therefore reductions in potassium conductances might be a ubiquitous component of hypertension.

The mechanisms underlying the observed reduction in the amplitude of TOCs in 4-wk HT rats are not known. The simplest explanation consistent with the lack of change in activation and deactivation kinetics is a reduction in the number of A-type potassium channels. The specific Kv channel subtype(s) that might be reduced in hypertension is/are not known. A-type K current amplitude in basal ganglia and basal forebrain neurons is linearly related to the abundance of Kv4.2 (Tkatch et al. 2000) and in neostriatal cholinergic interneurons to the abundance of Kv4.2 and Kv4.1 (Hattori 2003; Song et al. 1998). A-type K current in dopaminergic substantia nigra neurons is mediated by Kv4.3L (long splice variant) and KChip3 subunits (Liss 2001). Kv3.4 channels can also mediate A-type currents, and Kv3.4 immunoreactivity has been localized within the NTS (Brooke et al. 2004). Information on the expression of these and other potassium channel subunits in NTS neurons and the factors that regulate the expression of these subunits could provide insights into the mechanisms that might mediate changes in TOCs in hypertension. The potential mechanisms that might mediate reduced TOCs in NTS neurons in hypertension are numerous (altered levels of peripheral or central afferent input and/or neuronal discharge, circulating or local hormones) and beyond the scope of this study.

The observed reductions in TOCs showed some specificity for neurons receiving baroreceptor inputs because DiA-labeled neurons from 4-wk HT rats had a significantly shorter duration delayed excitation than DiA-labeled neurons from NT rats. This emphasizes the importance and necessity of some functional identification of the neurons being studied. DiA-labeled cells uniformly exhibited reduced amplitude TOC and increased duration of delayed excitation. The trends observed in the larger populations of unidentified neurons could likely be influenced by the relative presence or absence of neurons that undergo the same change as the DiA-labeled neurons. A recent study suggests that NTS neurons with prominent TOCs receive

C-fiber afferent inputs (Bailey et al. 2002). Lower density of TOC accelerates the rate at which excitation occurs in response to depolarizing inputs and may facilitate the occurrence of high-frequency discharge. An increase in the number of NTS neurons responding to aortic nerve stimulation with multiple spikes in renal wrap HT rats (Zhang and Mifflin 2000) has been reported. If TOCs contribute to frequency-dependent adaptation of afferent input, reduction in TOCs should increase ability of NTS neurons to follow high-frequency, C-fiber afferent inputs. It has been reported that C-fiber baroreceptor afferents reset less during renal hypertension (Thoren and Jones 1977). As a result of these two factors, the overall contribution of C-fiber afferents to baroreflex regulation may increase in HT relative to A-fiber inputs. In 4-wk HT rats, the baroreflex curve relating renal sympathetic nerve discharge to MAP is shifted to the right with no change in gain, while the curve relating HR to MAP is shifted to the right with a reduced gain (Vitela et al. 2005). The relative role of C-fiber versus A-fiber baroreceptor afferent inputs to specific aspects of reflex function remains to be determined.

ACKNOWLEDGMENTS

The expert technical assistance of M. Herrera-Rosales and O. Tolstykh is gratefully appreciated.

GRANTS

These studies were supported by National Heart, Lung, and Blood Institute Grant HL-56637.

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