Autoactivity of A5 neurons: role of subthreshold oscillations and persistent Na+ current

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Huangfu, Donghai, and Patrice G. Guyenet. Autoactivity of A5 neurons: role of subthreshold oscillations and persistent Na+ current. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2280–H2289, 1997.—A5 noradrenergic neurons play a key role in autonomic regulation, nociception, and respiration. The purpose of the present experiments was to characterize some of the intrinsic properties of A5 cells in vitro. Whole cell recordings were obtained from 85 spinally projecting neurons of the ventrolateral pons of neonate rats. Immunohistochemistry showed that 60% of the ventrolateral pontine cells were noradrenergic. Eighty percent of A5 neurons were spontaneously active (0.1–5.5 spikes/s). Their discharge rate, action potential duration, and, if so, to try and identify some of the underlying mechanisms.

METHODS

Whole cell recordings in the neonate slice. This work was carried out in thin slices from neonate rat brain, material that presents the advantage that individual cells can be visualized before whole cell recording (4). All recordings were obtained from cells retrogradely labeled with a fluorescent tracer injected into the spinal cord. This procedure was used to tag a cell population highly enriched in A5 noradrenergic neurons. The methods have been described in detail in previous papers dedicated to the study of C1 adrenergic neurons (16, 17). Briefly, Sprague-Dawley rat pups (3 days old) were anesthetized by deep hypothermia (24), and a suspension of fluorescein isothiocyanate (FITC)-labeled microspheres (0.3–0.5 µl; Molecular Probes) was injected bilaterally into the upper thoracic spinal cord. Two to seven days later, the pups (5–10 days old) were deeply anesthetized by hypothermia and decapitated. The brain stem was blocked and immersed in a sucrose-artificial cerebral spinal fluid (aCSF) mixture composed of (in mM) 26 NaHCO3, 1 NaH2PO4, 5 KCl, 5 MgSO4, 0.5 CaCl2, 10 glucose, and 248 sucrose and equilibrated with 95% O2-5% CO2 (pH 7.3). Coronal slices (130 µm thick) were cut with a microslicer (Dosaka), preincubated at 35°C for 30 min, and then brought to room temperature (22°C) in a lactic acid-aCSF mixture composed of (in mM) 124 NaCl, 26 NaHCO3, 5 KCl, 1 NaH2PO4, 2 MgSO4, 2 CaCl2, 10 glucose, and 4.5 lactic acid and equilibrated with 95% O2-5% CO2 (pH 7.3–7.4). For recording, a single pontine slice was placed in a recording chamber on an upright, epifluorescence microscope (Olympus BH-2). Slices located in immediate proximity to the exit point of the facial nerves were selected for recording. Slices containing the A5 region were identified under a ×10 objective by their characteristic pattern of retrograde labeling (for details, see Pattern of retrograde labeling in the ventrolateral pons of the neonatal rat). In this chamber, the slice was continuously superfused at the rate of 2–3 ml/min with normal aCSF equilibrated with 95% O2-5% CO2 (pH 7.4; composition identical to lactic acid-aCSF without the lactate). The reduced-Ca2+-aCSF used in some experiments contained 0.1 mM CaCl2 and 5 mM MgSO4. In other experiments, NaCl (124 mM) was replaced by an equimolar concentration of N-methyl-d-glucamine (NMDG) titrated with HCl. All experiments were performed at room temperature. Individual retrogradely labeled neurons were visualized with a water-immersion ×40 objective via epifluorescence and Hoffman modulation optics. Patch pipettes were

The cellular properties of A5 neurons have not been examined yet. The present study was designed to start filling this gap. The first objective was to determine whether A5 neurons possess characteristic electrophysiological properties that would permit their identification in vitro without having to resort to post hoc histology. The second main objective was to determine whether A5 neurons are autoactive like several other cell groups involved in sympathetic tone generation and, if so, to try and identify some of the underlying mechanisms.
pulled from borosilicate glass capillaries (1.5 mm OD; Clark) on a pipette puller (Sutter P87), coated with Sigmacote (Sigma Chemical, St. Louis, MO), and filled with a solution of the following composition (in mM): 144 K gluconate, 17.5 KCl, 4 NaCl, 4 MgCl2, 10 N-2-hydroxymethylperazine-N’-2-ethanesulfonic acid, 0.2 ethylene glycol-bis(β-aminoethyl ether)-N,N’-N’,N’-tetraacetic acid, 3 MgATP, and 0.3 NaGTP and 0.02% lucifer yellow (Molecular Probes). Osmolarity was adjusted to 270 mosmol, and the pH was adjusted to 7.3. Electrode resistance was 5–7 MΩ. Whole cell current- and voltage-clamp recordings were made with an Axoclamp-2A amplifier. The liquid junction potential was measured (8–12 mV), and all reported voltage measurements have been corrected for this potential. Series resistance compensation was not employed because the recorded currents were small enough (≤100 pA) that voltage errors due to series resistance should have been negligible. Current and voltage data were collected through a DigiData-1200 interface with pCLAMP software version 6.0 (Axon Instruments) and were stored on videotape for off-line analysis. Power spectrum density analysis was used to analyze membrane potential oscillations. In this case, membrane potential was sampled every 10 ms, and the power spectra represented averages of ten 20s segments. The SD of the membrane potential during a 100s segment (sampling every 10 ms) was also used to quantify membrane potential variability. In this case, the signal was filtered from 0.1 to 50 Hz because power spectrum density analysis revealed that the main density was distributed within this range.

Drugs and solutions of different ionic content were applied to the slice by switching the perfusion solution via a three-way electronic valve system. Time to onset of drug action was ~30 s.

After the recording was made, every slice was fixed in freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). Immunostaining for tyrosine hydroxylase (TH) was done with an avidin-biotin-based reaction (mouse-anti-TH monoclonal antiserum from Chemicon, dilution 1:1750; biotinylated goat anti-mouse antiserum from Vector, 1:150 dilution; avidin-conjugated Texas red from Molecular Probes, 1:200 dilution). Computer-assisted mapping of the location of retrogradely labeled, TH-immunoreactive (TH-ir), and/or lucifer yellow-stained neurons was done with a Ludl motor-driven stage and Neurolucida Software (MicroBrightfield, Colchester, VT). The atlas of Paxinos and Watson (23) was used for reference and nomenclature.

Drugs and chemicals. Tetrodotoxin (TTX), kynurenic acid, bicuculline methiodide, strychnine HCl, and NMDG were obtained from Sigma Chemical (St. Louis, MO).

Statistics. Results are expressed as means ± SE. Data were analyzed by either paired t-tests or analysis of variance. Significance was set at P < 0.05.

RESULTS

Pattern of retrograde labeling in the ventrolateral pons of the neonatal rat. These anatomic experiments were designed to determine the pattern of retrograde labeling of the ventrolateral pontine reticular formation in the neonate after injection of FITC-labeled microbeads into the thoracic spinal cord. The tracer was injected bilaterally into the upper thoracic spinal cord of three neonatal rats (3 days old). Two days later, serial 40-μm-thick coronal sections were cut with a vibratome throughout the rostral medulla and pons. A one-in-three series was processed for the immunofluorescent detection of TH, and adjacent sections were Nissl stained to add anatomic accuracy. As shown in Fig. 1 (left), the caudal pons is well differentiated at this early age. The distribution of spinally projecting neurons (with and without TH-ir) was mapped with the assistance of a computer. The number of each type of cell was then counted within a fixed-size rectangular window (0.58 × 0.53 mm) that was placed medial to the exiting root of the facial nerve over the area that contained the highest concentration of retrogradely labeled cells (Fig. 1). In this window, we counted 159 ± 18 TH-ir cells·animal−1·side−1, 63.5% of which (101 ± 10 cells) were retrogradely labeled with microbeads (examples of single and dual labeling are shown in Fig. 2). In the same area, 66.9% of all retrogradely labeled cells counted (100 ± 12 of 151 ± 8 cells·side−1·rat−1) were TH-ir. Note (Fig. 1, right, insets) that the proportion of TH-ir cells among retrogradely labeled neurons was highest laterally, in the immediate proximity of the ventral surface.

Location and phenotype of the recorded neurons. Whole cell recordings were obtained from a total of 85 spinally projecting neurons recorded in the region of the ventrolateral pons outlined in Fig. 1, insets. Sixty-three lucifer yellow-stained neurons were recovered after histology. All contained FITC-labeled microbeads. Sixty percent of the recovered cells (38 of 63) were TH-ir, i.e., A5 cells. An example of one recorded cell identified as an A5 neuron is shown in Fig. 3. The presence of FITC-labeled microbeads did not interfere with the detection of lucifer yellow (the two A5 cells shown in Fig. 3 had an equivalent amount of microbead labeling). Also, as illustrated in Fig. 3, the presence of the two latter markers did not interfere noticeably with the detection of TH-ir, which is especially intense in A5 neurons. The location of all recovered cells was mapped by computer and replotted on three standardized coronal sections and is represented in Fig. 4.

General electrophysiological properties of spinally projecting neurons in the ventrolateral pons. Monoaminergic neurons often exhibit an electrophysiological signature that is sufficiently distinct from that of other types of surrounding neurons to render post hoc histological identification unnecessary. Accordingly, the first objective of the present study was to determine whether A5 cells can be distinguished from the other spinally projecting neurons of the ventrolateral pons. However, as summarized in Table 1, both types of spinally projecting cells had similar general characteristics (discharge rate, mean interspike membrane potentials, conductance, spike amplitude measured from threshold, and width measured halfway between threshold and apex). The action potential threshold was close to −47 mV. All cells included in this study had action potential overshoots of at least +10 mV (+21 mV on average). The majority of the recorded cells (69 of 85) had a low level of spontaneous activity. This was true regardless of phenotype (79% of A5 cells, 88% of non-A5 cells; an example of an A5 neuron is shown in Fig. 5A). Slower cells fired irregularly and exhibited slow membrane oscillations. Faster cells (>2 Hz) had a more regular discharge pattern (Fig. 5A). Injection of a
hyperpolarizing current converted a regular firing pattern into an irregular one with slow oscillations (Fig. 5A). At rest, each pattern could be found in both TH-ir and nonnoradrenergic spinally projecting cells of the ventrolateral pons. Spontaneous action potentials were followed by 6–12 mV afterhyperpolarizations lasting for 100–250 ms and were best observed in slowly firing or silent cells. Afterhyperpolarizations merged into slow depolarizations in cells with firing rates of >2 spikes/s (Fig. 5A). Bursts of action potentials produced by injection of depolarizing current (1 s duration) were followed by a long-lasting hyperpolarization of relatively modest amplitude (<5 mV; Fig. 5A). In current clamp, inward rectification was typically observed regardless of cell type (e.g., A5 cell in Fig. 5B), and in voltage clamp, a slowly developing inward relaxation [hyperpolarization-activated current (Ih)-like current] was produced when the membrane potential was clamped below −80 mV (e.g., A5 cell in Fig. 5, C and D). A variable and generally low frequency of postsynaptic potentials (PSPs) was present, which, in some cases, contributed to the generation of action potentials. Figure 5E represents a case where PSP frequency was above average. One action potential was triggered by a PSP (Fig. 5Ea), and another action potential was triggered from a slow depolarization (Fig. 5Eb). Perfusion with a mixture of synaptic blockers including kynurenic acid (a nonspecific glutamate-receptor antagonist; 0.5 mM), bicuculline (a γ-aminobutyric acid receptor antagonist; 20 µM), and strychnine (a glycine receptor antagonist; 1 µM) eliminated observable PSPs (assessed during periods when membrane potential was hyperpolarized to about −70 mV; data not illustrated; note that the Cl− equilibrium potential equals −35 mV under our experimental conditions). This treatment did not significantly change the average
resting discharge rate of nine spontaneously active cells tested (sample includes seven histologically identified A5 cells; 2.5 ± 0.7 spikes/s before drug and 2.4 ± 0.8 spikes/s after 10-min perfusion with the drug mixture), although slight increases or decreases were found in a few cases. Minimal accommodation of firing was found when neuronal discharges up to 8–10 Hz were induced by injection of up to 30 pA of depolarizing current (n = 22 cells, including 18 identified A5 cells; an example of one A5 cell is shown in Fig. 6A). Accordingly, frequency-current curves were almost linear in the range of current tested.

Membrane potential oscillations. All slow-firing cells exhibited spontaneous membrane oscillations between action potentials (typical example in Figs. 5A and 7A, top trace), and the same pattern could be produced by injection of a small amount of hyperpolarizing current into more active cells (Fig. 5A). In all cases, when enough negative current was injected to eliminate the action potentials (mean membrane potential below −58 mV), all A5 cells (n = 38) and most others (18 of 22 non-A5 and 14 of 16 unrecovered) showed spontaneous membrane potential oscillations (example of one A5 cell in Fig. 7A, middle trace). These irregular oscillations were characterized by slow rising and falling phases that lasted 100–500 ms with an amplitude of 2–7 mV. These membrane oscillations were voltage dependent and disappeared when enough hyperpolarizing current was injected to bring the membrane potential down to −65 mV or lower (Fig. 7A, bottom trace). The voltage dependency of the oscillations was quantified by mea-

Fig. 2. Retrograde labeling in coronal section (thickness 40 µm) of ventrolateral pons. A: TH-ir. B: fluorescent microbeads. Arrows, retrogradely labeled TH-ir neurons. *Retrogradely labeled cells devoid of TH-ir. Bar, 100 µm.

Fig. 3. Recorded A5 neuron. A: lucifer yellow-labeled neuron (ultraviolet incident light, fluorescein isothiocyanate filter). B: same field under green light illumination to reveal TH-ir (Texas red filter). Note presence of a 2nd spinally projecting TH-ir cell that was not recorded. C: higher power photograph of recorded cell under the same illumination condition as in A to reveal microbeads. Bars: 25 µm in A and B; 10 µm in C.
Contribution of Na⁺ currents to the membrane oscillations. Three types of experiments were performed to test whether the membrane oscillations were due to a TTX-sensitive (TTXₜ) persistent Na⁺ current (I₆Na). The first experiment tested the effect of NMDG (extracellular Na substitution) on the magnitude of the oscillations. In the second experiment, we searched for the presence of a persistent voltage-activated TTXᵢ inward current using a classic voltage-clamp paradigm (19). Finally, we determined whether the membrane oscillations were sensitive to TTX.

Perfusion with a medium in which 82% of the Na⁺ was replaced with NMDG produced a 1- to 5-mV hyperpolarization within 1–2 min (Fig. 8A). The low-Na⁺ medium virtually eliminated the membrane oscillations even after the membrane potential had been restored to its original level (Fig. 8B). In a group of nine cells, the SD of the membrane potential was significantly reduced by NMDG (Table 2). The mean membrane potential at which the measurements were made did not differ (Table 2). The oscillations returned within 2 min after reperfusion with control aCSF (Fig. 8B). To determine whether the membrane fluctuations were periodic, we performed a power spectral analysis of the membrane potential of eight of these nine cells (sample included six A5 cells). In normal saline, most of the power was found below 6 Hz. In most cells (5 of 8; Fig. 8C), the power spectrum exhibited a large peak at 0.9–1 Hz (49,000 ± 5,900 mV²) and two smaller peaks at −0.5 (29,200 ± 6,100 mV²) and 1.6–2.0 Hz (21,600 ± 4,500 mV²). In the other three cases, the peak at 0.5 Hz was larger than the one at 1 Hz (44,200 ± 6,800 vs. 35,800 ± 6,200 mV²). In the presence of NMDG, the total power (integrated between 0.1 and 10 Hz) was dramatically reduced as illustrated in Fig. 8C. On average, the total power was reduced 8.5-fold by NMDG (from 0.119 ± 0.015 to 0.014 ± 0.004 V²·Hz; P < 0.05; n = 8 cells).

To test for the presence of voltage-activated persistent I₆Na, the membrane potential was stepped for 200 ms from a holding level of −65 mV to more depolarized potentials (−60, −55, and −50 mV; Fig. 9Aa). The peak inward current observed with the most depolarized step was always <100 pA. The step paradigm was repeated after application of 0.1 µM TTX. Then, the toxin was washed until complete recovery (10 min), and

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Resting Membrane Potential, mV</th>
<th>Conductance, nS</th>
<th>Amplitude, mV</th>
<th>Width, ms</th>
<th>Firing Rate, spikes/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>All spinally projecting cells</td>
<td>−57.4 ± 0.7</td>
<td>1.82 ± 0.12</td>
<td>−69.3 ± 0.9</td>
<td>3.68 ± 0.09</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>(25; 43–73)</td>
<td></td>
<td></td>
<td>(69.55–82)</td>
<td>(69.25–5)</td>
<td>(69.01–10)</td>
</tr>
<tr>
<td>A5 cells</td>
<td>−56.6 ± 1.0</td>
<td>1.71 ± 0.13</td>
<td>−66.6 ± 1.3</td>
<td>3.98 ± 1.13</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>(38; 43–69)</td>
<td></td>
<td></td>
<td>(30; 55–80)</td>
<td>(30; 2.5–5)</td>
<td>(30.01–5.5)</td>
</tr>
<tr>
<td>Non-A5 cells</td>
<td>−58.4 ± 1.4</td>
<td>1.73 ± 0.22</td>
<td>−69.6 ± 1.5</td>
<td>3.41 ± 1.13</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>(25; 48–73)</td>
<td></td>
<td></td>
<td>(22; 55–80)</td>
<td>(22; 2.5–5)</td>
<td>(22; 0.1–10)</td>
</tr>
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Values are means ± SE; nos. in parentheses, no. of neurons; range of data. A5 cells, histologically recovered tyrosine hydroxylase-immunoreactive (TH-ir) neurons; non-A5 cells, histologically recovered non-TH-ir cells. Resting membrane potential, mean interspike potential in slow cells or average between afterhyperpolarization nadir and spike threshold for faster cells.
the preparation was perfused with low-Na medium (23 mM Na). The threshold for producing an inward current was typically between -60 and -55 mV (an example of an A5 cell is shown in Fig. 9Aa). Both the early and the persistent current were eliminated by 0.1 µM TTX. Figure 9Ab illustrates the current recorded in the presence of TTX, and Fig. 9Ac illustrates the difference current (control current minus residual current in the presence of TTX). The inward current was also eliminated by lowering Na (Fig. 9Ad). This experiment was carried out in a total of eight A5 cells and in four noncatecholaminergic neurons. Seven of eight A5 cells and two of four non-A5 cells responded as illustrated in Fig. 9A. In one A5 cell, only the persistent component of the current was observed, and it was obliterated by TTX. In two of the noncatecholaminergic cells, only the transient inward current was present.

To determine whether the TTX inward \( I_{Na} \) contributes to the membrane potential oscillations of A5 cells, 17 cells were recorded in current-clamp mode, and we determined the effect of 1 µM TTX (10-min exposure) on the SD of the membrane potential. TTX typically reduced but did not eliminate the oscillations (Fig. 9B). This reduction was significant (Table 2). TTX produced no consistent change in resting membrane potential and eliminated the action potentials elicited by a depolarizing current injection (data not shown).

**DISCUSSION**

This study is the first to describe the cellular properties of A5 neurons in vitro. Although spinally projecting neurons were selectively recorded, this population is probably a fair representation of the entire A5 cell group given that the majority of A5 neurons (up to 75% in one rat) have an axonal projection to the spinal cord. The present study was done in the neonate because the optical properties of neonatal brain slices permit individual neuronal cell bodies to be seen and patched with relative ease. This is not the case in adult tissue due to the development of myelin. The results of the present study may not be strictly applicable to adult A5 neurons.

General properties of A5 and non-TH-ir spinally projecting neurons. Monoaminergic neurons often exhibit an electrophysiological signature that is sufficiently distinct from that of other types of surrounding neurons to render post hoc histological identification unnecessary. For example, the dopaminergic neurons of the midbrain can be identified by a combination of features that include broad action potentials, spontaneous activity, and a large time- and voltage-dependent inward current \( I_{h} \) (5). These characteristics have diagnostic value even in thin slices of neonatal brain (27). In the present case, we found no obvious electro-
physiological difference between A5 neurons and the rest of the surrounding spinally projecting cells except for the fact that A5 cells were selectively responsive to \( \alpha_2 \)-adrenergic stimulation [see companion paper (6a)].

A5 and non-TH-ir spinally projecting cells had similar discharge rates at rest, a similar input resistance, and equally broad action potentials. Their \( I_h \)-like current was unremarkable, and their accommodation properties were the same. It is unlikely that many of the neurons classified as noncatecholaminergic could have been A5 cells exhibiting lower than normal levels of TH-ir. One reason is that the TH-ir of A5 cells is very intense and easy to detect (Fig. 3). The second is that, even under optimal staining conditions (thin sections prepared exclusively for histology), the A5 region contains spinally projecting cells that are clearly not TH-ir (see Figs. 1 and 2). Finally, the cells that were not TH-ir seemed as healthy as the A5 cells (Table 1). On the basis of the results of recent viral retrograde tracing data, it is possible that some of the nonnoradrenergic cells might also innervate sympathtic preganglionic neurons (10).

The general properties of A5 cells were similar to those of the C1 adrenergic neurons previously recorded under similar conditions (16). The resemblance includes all of the basic parameters summarized in Table 1: the minimal accommodation of firing, the generally modest inward rectification, and the presence of slow and irregular membrane oscillations from which the action potentials of the slower firing cells appear to be triggered. Autoactivity in vitro is by no means a unique characteristic of the catecholaminergic neurons and is found in many other brain stem neurons (e.g., Refs. 11, 22).

Table 2. Properties of subthreshold oscillations

<table>
<thead>
<tr>
<th>Experiment</th>
<th>n</th>
<th>MP, mV</th>
<th>MP Variability, mV</th>
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<tr>
<td>Control*</td>
<td>17</td>
<td>-59.2 ± 1.8</td>
<td>0.92 ± 0.06</td>
</tr>
<tr>
<td>Hyperpolarized</td>
<td>17</td>
<td>-58.2 ± 1.5</td>
<td>0.88 ± 0.06</td>
</tr>
<tr>
<td>Low Ca(^{2+})-high Mg(^{2+})</td>
<td>9</td>
<td>-58.5 ± 1.9</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td>Normal sodium*</td>
<td>7</td>
<td>-58.3 ± 2.4</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>NMDG†</td>
<td>7</td>
<td>-59.3 ± 2.0</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>Normal aCSF*</td>
<td>7</td>
<td>-59.2 ± 2.0</td>
<td>0.35 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, total no. of cells; nos. in parentheses, no. of histologically identified A5 cells. MP, membrane potential; aCSF, artificial cerebrospinal fluid; NMDG, N-methyl-D-glucamine; TTX, tetrodotoxin; NS, not significant. *Cells slightly hyperpolarized to eliminate spontaneous action potentials; †MP deliberately restored to original level by intracellular current injection.
Autoactivity of A5 neurons. The generally slow and irregular discharges of A5 cells were most likely due to intrinsic cell properties for the following reasons: PSPs were infrequent, and the discharge rate of the cells was not changed significantly by adding a mixture of synaptic blockers that eliminated detectable synaptic activity (kynurenate, strychnine, and bicuculline). In addition, in the slower cells, action potentials appeared to be triggered from slow membrane depolarizations. These membrane depolarizations were probably not due to synaptic activity because they were unaffected by synaptic blockade with reduced extracellular Ca\(^{2+}\) (Table 2).

Possible mechanisms underlying the membrane potential oscillations of A5 neurons. The power spectrum of the membrane potential of A5 neurons displayed one or more peaks between 0.5 and 2 Hz (Fig. 8), suggesting that the membrane potential fluctuations were not random. However, the power was rather broadly distributed in the 1- to 8-Hz range, consistent with the fact that the oscillatory behavior of A5 cells is not as regular or as large as in many other types of neurons (e.g., Refs. 12, 18, 21). In other systems, the frequency of neuronal oscillations increases steeply, with the membrane potential in the range of \(-60\) to \(-50\) mV (12, 18). The low frequency found in A5 cells may reflect the fact that the membrane oscillations were examined while the membrane potential was maintained close to \(-60\) to \(-55\) mV to prevent action potential generation.

The most common mechanisms of membrane potential oscillations involve some form of voltage-activated \(I_{Na}\) (e.g., Ref. 18) and or a low-threshold Ca\(^{2+}\) current (e.g., Refs. 12, 14). Because 1 µM TTX reduced the amplitude of the oscillations significantly (Table 2), a TTX-sensitive persistent inward current contributes to the phenomenon (30). Voltage-clamp experiments such as the one shown in Fig. 9 suggest that A5 neurons may have a TTX-sensitive persistent inward current that activates close to the mean resting potential of \(-60\) to \(-55\) mV (Fig. 9). This interpretation is based on the assumption that the persistent component of the TTX-sensitive inward current was not an artifact due to a poor space clamp in dendrites (29). The TTX-sensitive \(I_{Na}\) is thought to play a role in the oscillatory behavior of many central nervous system neurons (e.g., Refs. 14, 19). Its origin is attributed to one of three possible mechanisms: a window current due to the overlap between the activation and inactivation properties of the transient Na\(^{+}\) channels, a current generated by channels distinct from the latter, and a
modal change in the inactivation properties of the transient Na\(^+\) channels (for a review, see Ref. 3).

Because a large part of the subthreshold oscillations of A5 neurons typically persisted in the presence of 1 µM TTX (Fig. 9, Table 2), TTX\(_r\) I\(_{\text{Na}}\) appears to amplify membrane oscillations caused by other types of conductances. These conductances are also presumably activated by depolarization because the oscillations were severely attenuated by holding the cell soma at or below −65 mV (Fig. 7, Table 2). Two possible candidates are a low-threshold Ca\(^{2+}\) conductance and a TTX-insensitive (TTX\(_r\)) I\(_{\text{Na}}\) (for a review, see Ref. 30). Because all the inward current recorded during a step depolarization to −50 mV was sensitive to 0.1 µM TTX (Fig. 9), this voltage-clamp protocol did not provide evidence for either of the two possibilities. Perhaps this negative result indicates that the voltage-activated conductances responsible for the membrane oscillations reside primarily in distal dendrites. The TTX\(_r\) conductances responsible for the membrane oscillations observed in the present study. In addition, these membrane oscillations would be less apt to trigger action potentials if the cells had more negative resting potentials. Whether adult A5 neurons have the proper mix of persistent current, input resistance, and resting membrane potential required for spontaneous action potential generation, especially in vivo, needs to be investigated.

Functional significance. Sympathetic vasomotor tone depends on a relatively small number of spinally projecting, monosynaptic, mostly excitatory inputs to sympathetic preganglionic neurons (presympathetic neurons) (9). Contrary to the motoneuronal outputs to skeletal muscles, the sympathetic vasomotor outflow is very resistant to anesthesia. One possible explanation for this resistance is that under anesthesia the discharges of brain stem presympathetic neurons depend minimally on synaptic inputs because the cells have autovagogenic properties. Previous work (16, 17) has suggested that the C1 adrenergic cells and other nonadrenergic cells of the rostral ventrolateral medulla may have such characteristics. The present work adds the A5 neurons to the list of spinally projecting presympathetic neurons that may have autovagogenic properties. More work is needed to verify that the autovagogenia of A5, C1, and many other medullary cells recorded in the neonate persists in the adult.

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