Electrophysiological properties of rat lateral parabrachial neurons in vitro

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Hayward, Linda F., and Robert B. Felder. Electrophysiological properties of rat lateral parabrachial neurons in vitro. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R696–R706, 1999.—Anatomical studies have demonstrated that the lateral parabrachial nucleus (LPBN) is composed of at least seven separate subnuclei distinguished by cell morphology, spatial clustering, and afferent and efferent connectivity. We hypothesized that neurons within the subnuclear clusters of the LPBN might have distinct electrophysiological properties that correlate with cellular morphology. An in vitro slice preparation was used to intracellularly record the intrinsic properties of 64 neurons located within the external lateral (EL) and central lateral (CL) subnuclei of the LPBN in adult rats. Analysis of intrinsic properties revealed that neurons in the EL subnucleus had significantly wider action potentials and on the average demonstrated more spike frequency adaptation during 2 s of depolarization compared with CL neurons. The majority of both EL and CL area neurons expressed delayed excitation (DE) after membrane hyperpolarization. DE was eliminated with the A-current blocker 4-aminopyridine (1.5–5 mM). Postinhibitory rebound was also observed in a subpopulation of EL and CL neurons. Morphological analysis of 11 LPBN neurons, which were electrophysiologically characterized and filled with 2% biocytin, failed to demonstrate an association between morphology and the electrophysiological profiles of LPBN neurons. The lack of distinct “type” of neuron within a single subnucleus of the LPBN is in agreement with recent findings reported from the neonatal rat.

lateral parabrachial nucleus

THE LATERAL PARABRACHIAL nucleus (LPBN) is located in the dorsolateral pons and is composed of at least seven distinct subnuclei, distinguished by cell morphology and spatial clustering. Because anatomical studies have identified the LPBN as a major ascending projection site for neurons located in the cardiovascular region of the caudal dorsomedial nucleus of the solitary tract (NTS), there has been increased interest in the function of the LPBN in cardiovascular regulation. Stimulation and lesion studies have demonstrated that the LPBN is involved in a variety of physiological functions from the neurohumoral control of the circulation to the modulation of respiratory and pain responses (3, 22, 24, 26). Some of the complexity of the LPBN may be explained by this subnuclear organization. For example, in addition to being morphologically distinct, each of the seven subnuclei in the LPBN have relatively specific anatomical interconnections (12, 13, 18).

Although anatomical studies have defined the afferent and efferent interconnections of LPBN subnuclei, less is known about the synaptic connections, the neurotransmitter mechanisms, and the intrinsic cellular properties of LPBN neurons, all of which can strongly influence afferent processing and the pattern of neuronal output. We hypothesized that neurons within the subnuclear clusters of the LPBN might have distinct electrophysiological properties that correlate with cellular morphology. Although investigations of other central nuclei have demonstrated such correlations between intrinsic electrophysiological properties, neuronal morphology, and subnuclear organization (9), a detailed analysis of LPBN neurons in relationship to their subnuclear location and morphology has not been undertaken. The present study was designed to examine in detail the intrinsic properties of the neurons located within the different subnuclei of the LPBN. Because one of the better-defined functions of the LPBN at this time is in cardiorespiratory regulation, we focused on electrophysiologically characterizing neurons in two subnuclei previously identified to receive inputs from the caudal NTS, the primary central termination site for cardiorespiratory afferents (i.e., baroreceptor, chemoreceptor, and vagal afferents; see Ref. 18). These two subnuclei include the central lateral (CL) and the external lateral (EL) subnuclei of the LPBN. Preliminary results of this work have been presented (17).

METHODS

Adult male Sprague-Dawley rats (200–300 g) were anesthetized with halothane and decapitated. The brain was quickly removed and placed in an oxygenated modified (m) artificial (a) cerebrospinal fluid (CSF) solution cooled to 3–5°C. The m-aCSF solution consisted of (in mM) 125 NaCl, 5 KCl, 1.3 MgSO4, 2.4 CaCl2, 2.5 NaH2PO4, 125 NaHCO3, 1.25 NaH2PO4, and 10 glucose (1). After 1 min of cooling, the brain was removed and trimmed to an ~1 × 0.5 × 0.4 cm block of the rostral pons containing the LPBN, identified by the presence of the superior cerebellar peduncle just caudal to the inferior colliculus. The tissue was then attached to a Teflon block with cyanoacrylate glue and submerged in the cooled oxygenated m-aCSF. The rostral pons was then sliced into 400-μm-thick transverse sections with a vibratome (Campden Instruments). Two to three transverse slices of the rostral pons were placed in a standard recording chamber, submerged, and perfused at a rate of 2 ml/min by gravity-fed oxygenated aCSF containing (in mM) 125 NaCl, 5 KCl, 1.3 MgSO4, 2.4 CaCl2, 2.5 NaH2PO4, 125 NaHCO3, and 10 glucose (pH 7.4 after

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saturation with 95% O₂-5% CO₂) warmed to 34 ± 2°C. The majority of the slices were subfused, and humidified 95% O₂-5% CO₂ was continuously blown across the surface of the slice. For those experiments in which 4-aminopyridine (4-AP; Sigma) was applied to the bath solution, the slices were superfused and held in place under a small grid of parallel nylon threads glued to a U-shaped piece of platinum wire (WPI). All slices were allowed at least 1 h of equilibration before recording commenced. Slices remained viable for 4–7 h.

Intracellular recordings were obtained with glass microelectrodes (ID 0.5 mm; Sutter Instruments) pulled on a Flaming-Brown P-87 micropipette puller (Sutter Instruments) and filled with either 3 M KCl (impedance 80–120 MΩ) or 2% biocytin (Sigma, St. Louis, MO) in 1 M KCl and 0.05 M Tris. The microelectrode was connected to the head stage of an Axoclamp 2A amplifier (Axon Instruments), and intracellular recordings were made in either the bridge, discontinuous current clamp (DCC), or single electrode voltage clamp (SEVC) mode. The bridge balance was continuously monitored on an oscilloscope (Tektronix) to measure the voltage drop across the electrode. In DCC or SEVC mode, the switching frequency was 3.5–5 kHz (50% duty cycle). The head stage output was continuously monitored on a second oscilloscope to ensure adequate adjustment of the capacitance feedback. Current and voltage data were recorded with a PCM recording adapter (Vetter) and a VCR for later analysis.

4-AP was applied to the slice by turning a stopcock and interrupting flow of the normally perfused, gravity-fed aCSF and switching the perfusion solution to an oxygenated, warmed aCSF containing 4-AP (1.5–5 mM). Complete exchange of the bathing solutions took 2–3 min.

Experimental protocol. Recording electrodes were positioned on the surface of the slice in the region of either the EL or CL subnuclei of the LPBN with the aid of an operating microscope (Nikon). The electrode was then advanced through the tissue using a microdrive (Kopf model 660, Tujunga, CA). The subnuclei were defined by their relationship to the brachium conjunctivum, which was easily visualized under the microscope. Single neurons with resting membrane potentials (RMP) of at least −50 mV, action potentials with amplitudes >65 mV, input resistances (Rᵢ) >50 MΩ, and stable impalement times of at least 15 min were selected for study.

Action potentials were recorded in the bridge mode for analysis. Action potentials were either spontaneously occurring or were elicited by injection of a small amount of depolarizing current. Hyperpolarizing current pulses applied in the DCC mode were used to measure membrane Rᵢ and membrane time constant. Current pulses (2-s duration pulses) of increasing amplitude were applied until the hyperpolarized potential reached −100 to 110 mV. In some cells, ramp increases in voltage (−100 to −40 mV) were performed in the SEVC mode to further characterize the current-voltage relationship.

Repetitive firing properties of single neurons were characterized by applying depolarizing current pulses of varying amplitude (2-s duration pulses) in the bridge mode until a rapid steady-state discharge rate was observed or the depolarized membrane potential (V_m) approached −20 mV. Peak and steady-state spike frequencies were then used to calculate spike frequency adaptation (SFA). Delayed excitation (DE), the delay of the onset of action potentials due to membrane hyperpolarization before depolarization, was elicited by applying hyperpolarizing pulses of increasing amplitude (constant duration, 2 s) in the DCC mode immediately after a positive pulse (0.01–0.2 nA, 2 s). Increasing levels of hyperpolarizing current were injected until a maximum hyperpolarization of −110 mV was achieved. If there was evidence of a delay, then the effect of the duration of hyperpolarization was tested by applying hyperpolarizing pulses of a constant amplitude (typically sufficient current to hyperpolarize to −100 mV) and increasing duration (100–2,000 ms). The presence of postinhibitory rebound (PIR) was identified by a rebound firing of several action potentials at the offset of membrane hyperpolarization (0.01–0.2 nA, 2 s) and the return to RMP.

Biocytin staining. After successful impalement and electrophysiological characterization of several LPBN neurons, biocytin was iontophoretically injected for recovery of the morphology of the neuron. Biocytin was injected by anodal current pulses (200 ms at 1 Hz, 0.2–0.5 nA, sufficient to lower V_m to −50–50 mV) for 10–20 min. At the end of the experiment, the brain slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, and refrigerated overnight. One or two days later, the tissue was glued to the cutting stage of the vibratome, submerged in 0.01 M phosphate-buffered saline (PBS), and resected in 100-μm slices. The slices were then mounted on a gelatin-coated slide and incubated in 0.1 M PBS containing 0.1% Triton X and fluorescein isothiocyanate (FITC)-conjugated avidin (Jackson Immunol. Research) at room temperature for 2–4 h. The slides were then rinsed in 0.01 PBS and covered with Vectashield (Vector Labs) to prevent drying and photobleaching. The biocytin-labeled neurons were identified with a light microscope (Nikon Labophot) equipped with epifluorescence and a side arm. Camera lucida drawings were made of the FITC-labeled neurons and their location within the LPBN.

Data analysis. All data were analyzed off-line using a Cambridge Electronics Design (CED, Cambridge, UK) 1401 Laboratory Interface coupled to a Gateway 486 computer and CED software. Rᵢ was determined in all neurons by dividing the change in V_m by the magnitude of hyperpolarizing current pulses that caused a voltage deflection between 10 and 25 mV. The average of several of these pulses was reported as the Rᵢ for each neuron. In a subpopulation of LPBN neurons, Rᵢ was also calculated from the linear relationship between current intensity and V_m displacement measured from −7 to −12 hyperpolarizing pulses of increasing amplitude in the DCC mode, or input conductance was calculated from the current and voltage changes in the SEVC mode at a rate of 1 kHz during a ramp increase in voltage from −100 to −40 mV. In these two instances, Rᵢ or conductance was calculated using the linear portion of the current-voltage plot using linear regression analysis. Membrane time constant was determined from the exponential decay of the voltage response to a single hyperpolarizing pulse (0.1–0.2 nA) in the DCC mode. Voltage data was sampled at 1-µs intervals.

The amplitude of action potentials was measured with respect to the RMP. Action potential duration was calculated as the duration measured at the half amplitude. The maximum dV/dt (change in voltage with respect to time) of the rising and falling phases of the action potentials was determined from the first derivative of the action potential voltage. Action potential afterhyperpolarization (AHP) amplitude was measured with respect to the action potential threshold. AHP duration was calculated from the time the V_m dropped below the RMP after the peak voltage of the action potential to the time V_m returned to RMP.

All data are expressed as means ± SE. Statistical analysis was carried out by either cross-correlation analysis (Pearson product-moment correlation coefficient) or an ANOVA with post hoc comparison between groups evaluated by a Scheffé F-test (StatView); P < 0.05 was considered significant.
RESULTS

Resting intrinsic membrane properties. Stable intracellular recordings were made from 64 neurons located within the LPBN, including 42 neurons recorded from the EL region and 22 neurons recorded from the CL region. The majority (80%) of all LPBN neurons were silent after impalement and membrane stabilization, although 13 of 64 neurons had spontaneous activity. Six of the spontaneous neurons were located in the EL subnucleus, and seven were located in the CL subnucleus. There were no significant differences in the mean spontaneous discharge rate of neurons located in the EL versus the CL subnucleus (1.2 ± 0.2 vs. 0.4 ± 0.2 spikes/s, EL vs. CL, respectively). Analysis of neuronal intrinsic properties identified no significant differences in the RMP or membrane time constant between neurons located in the EL versus the CL subnuclear regions (see Fig. 1). During injection of hyperpolarizing current pulses, 22 of 64 LPBN neurons showed evidence of an initial slow repolarization of V_m at the onset of hyperpolarization. This time-dependent anomalous rectification was observed in 15 EL and 7 CL neurons (see Fig. 1) and was most apparent during membrane hyperpolarization to levels less than −90 mV. The relationship between the amplitude of negative current injected and the steady-state V_m (measured during the last 500 ms of hyperpolarization) was linear for all LPBN neurons during membrane hyperpolarization less than −100 mV (see Fig. 1) and there was no significant difference between the mean R_m of neurons located in the EL versus the CL subnuclei (137 ± 8 vs. 125 ± 14 MΩ, EL vs. CL, respectively; P < 0.4).

Action potential characteristics. Measurement of action potential characteristics evoked by minimal step depolarizations from RMP demonstrated no significant difference in action potential amplitude (75.5 ± 1 vs. 74 ± 2 mV, EL vs. CL, respectively) or the maximum rate of rise (205 ± 20 vs. 234 ± 23 mV/ms, EL vs. CL, respectively) of the action potentials associated with neurons in the EL versus the CL subnuclei (see Fig. 2). There was however a significant difference in the action potential width and maximum rate of action potential decay (see Fig. 2, B and D). Neurons from the CL region of the LPBN had significantly shorter action potential widths and significantly faster rates of decay (−94 ± 7 vs. −127 ± 16 mV/ms, EL vs. CL, respectively; P < 0.03). Correlation analysis, however, revealed no significant relationship between action potential characteristics (width, rate of rise/fall) and RMP or R_m (r < 0.08).

Repetitive discharge characteristics. Two second depolarizing current pulses of varying amplitude evoked two main patterns of repetitive discharge in all LPBN neurons. The first pattern observed in some LPBN during depolarization was characterized by a short burst of action potentials at the onset of depolarization followed by rapid SFA (see Fig. 3A). Increased depolarization resulted in a more rapid initial burst of action potentials, but steady-state discharge continued to be slow or unsustained. The second pattern of discharge evoked in other LPBN neurons was characterized by continuous discharge for the duration of the depolarizing pulses (see Fig. 3B). The relationship between the peak instantaneous firing frequency (peak f; typically calculated from the interval between the first and second spike) and the amplitude of the positive current injected was linear for all LPBN neurons within the range tested (0.05–0.5 nA; see Fig. 3, C and D). The slope of the relationship between current and the steady-state f (averaged during the last 500 ms of 2-s depolarizing pulses) was also linear for all neurons that maintained a discharge during the last 500 ms of
depolarization. Cross-correlation analysis of all neurons demonstrated no significant relationship between either the slope of the peak current-iF relationship or the slope of the steady state current-iF relationship and RMP, membrane R_N, or action potential width (r < 0.4). There was also no significant difference in the mean slope of the peak current-iF relationship (232 ± 23 vs. 194 ± 32 spikes·s⁻¹·nA⁻¹, EL vs. CL, respectively) or the mean slope of the steady-state current-iF relationship between neurons located in the EL versus the CL region of the LPBN, although there was a trend for the slope of the relationship for neurons in the CL subnucleus to be slightly higher (41 ± 5 vs. 66 ± 15 spikes·s⁻¹·nA⁻¹, EL vs. CL, respectively; P > 0.06).

The percent adaptation of the steady-state current-iF relationship from the peak current-iF curve was calculated for all neurons tested. The percent SFA ranged from 100% (no sustained discharge) to only 15% rate adaptation over 2 s of discharge. Figure 4A illustrates the distribution of percent SFA for all neurons (EL vs. CL). Correlation analysis of all neurons demonstrated no correlation between the percent SFA and RMP, R_N, action potential width, or action potential rate of fall (r < 0.26). However, comparisons of the

Fig. 2. Action potential characteristics of EL vs. CL parabrachial neurons. A: two typical action potential profiles recorded from EL vs. CL parabrachial nucleus (PBN) neuron. B: comparisons of mean action potential widths of all neurons located in EL vs. CL subnucleus of PBN. C and D: comparison of range and distribution of rate of action potential rise (C) and fall (D) measured from all EL vs. CL neurons. *Significant difference between groups (P < 0.05).

Fig. 3. Repetitive firing patterns recorded from two different EL neurons. A: step membrane depolarization of one neuron resulted in a brief burst of action potentials that was not sustained. PTH, posttetanic hyperpolarization; DE, delayed excitation. B: step depolarization of second neuron resulted in a brief rapid burst of action potentials that slowed to a steady continuous discharge rate. Greater depolarization of same neuron increased both rate of initial rapid burst of action potentials and steady-state discharge rate. C: plot of peak and steady-state instantaneous firing frequency (iF) plotted as a function of current (I) for rapidly adapting neuron in A. Lines represent a best fit by linear regression. Slopes of peak iF and steady-state iF relationship were 67 spikes/nA (r = 0.82) and 29 spikes/nA (r = 0.81), respectively. D: plot of the peak and steady-state instantaneous firing frequency (iF) plotted as a function of I for the slowly adapting neuron in B. Slopes of the peak iF and steady-state iF relationship were 347 spikes/nA (r = 0.92) and 77 spikes/nA (r = 0.99), respectively. Recordings were made in the bridge mode. Calibration pulse is applicable for A and B.
means showed that neurons located in the EL subnucleus had significantly greater percent SFA compared with those neurons located in the CL subnuclear region (85 ± 2 vs. 65 ± 7%, respectively; P < 0.009). Figure 4C illustrates the distribution of those neurons with 90% or greater SFA versus those neurons with <90% SFA. The CL subnucleus tended to contain primarily neurons with SFA <90%, whereas neurons in the EL subnucleus expressed more of a variety of SFA patterns. A comparison of the intrinsic membrane properties of those neurons with SFA ≥90% versus those neurons with <90% SFA demonstrated no significant differences between the two groups in AP height, AP width, RMP, R, or membrane time constant.

Immediately after the offset of the 2-s depolarizing pulses, there was evidence of posttetanic hyperpolarization in most neurons (PTH, see Fig 3). The peak amplitude and duration of the PTH increased when the neurons were depolarized to a higher level, evoking a higher peak firing rate (see Table 1). No significant difference between the mean amplitude or duration of PTH evoked in EL neurons versus CL neurons, however, was observed.

DE. In 44 of 62 neurons tested, prior membrane hyperpolarization resulted in a delay in the onset of discharge or DE. The duration of the DE was dependent on both the amplitude (see Fig. 5, A and C) and the duration of the preceding hyperpolarization (see Fig. 5, B and D), suggesting the delay was a manifestation of A-current. Neurons displaying DE included 27 of 41 EL neurons (65%) and 17 of 21 CL neurons (81%). Thirteen of these 44 neurons also displayed a delay in the onset of action potential discharge when depolarized from RMP. These 13 neurons included 11 EL neurons and 2 CL neurons. The mean delay in action potential generation after depolarization from rest for these 13 neurons was 130 ± 20 ms (range 27–269 ms). Comparisons between those neurons expressing DE at rest versus those neurons only expressing DE after hyperpolarization (n = 31) and those neurons in which DE was not expressed (n = 18) demonstrated that neurons expressing DE at rest had significantly lower RMPs than both neurons in which DE could be evoked and neurons not expressing any DE (see Fig. 6A). There was also a trend for the R of those neurons expressing DE at rest to be lower that the R of both neurons in which DE could be evoked and neurons not expressing DE (P = 0.09; see Fig. 6B). There was, however, no significant difference in mean action potential width, membrane time constant, or percent SFA between these three groups of neurons.

For all neurons expressing DE, the mean duration of the delay in action potential discharge after membrane hyperpolarization to a common level (–84 ± 2 mV) was 325 ± 30 ms. There was no significant correlation between the duration of DE and R, RMP, or percent SFA (r < 0.2). There was also no significant difference between the mean duration of DE evoked by a common level of membrane hyperpolarization in EL versus CL neurons (2-s hyperpolarization at –84 ± 1 mV elicited a delay of 265 ± 35 ms in EL neurons vs. 272 ± 56 ms in CL neurons). However, comparisons between the dura-

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<th>Cell Type</th>
<th>Mean Lower Firing Frequency, spikes/s</th>
<th>PTH Amplitude at Low Frequency, mV</th>
<th>PTH Duration at Low Frequency, ms</th>
<th>Mean Higher Firing Frequency, spikes/s</th>
<th>PTH Amplitude at Higher Frequency, mV</th>
<th>PTH Duration at Higher Frequency, ms</th>
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<tr>
<td>EL</td>
<td>13 ± 1</td>
<td>5 ± 0.5</td>
<td>485 ± 45</td>
<td>32 ± 2</td>
<td>7 ± 0.6</td>
<td>627 ± 69</td>
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<td>CL</td>
<td>14 ± 1</td>
<td>4 ± 0.7</td>
<td>393 ± 51</td>
<td>32 ± 3</td>
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n, no. of rats. PTH, posttetanic hyperpolarization; EL, external lateral; CL, central lateral.
Intrinsic Properties of Lateral Parabrachial Neurons

In addition to producing DE, membrane hyperpolarization immediately before depolarization also resulted in a noticeable slowing of the steady-state discharge in 26 of 44 LPBN neurons expressing a sustained discharge during 2 s of depolarization alone (including 20 EL and 6 CL neurons). This steady-state slowing was most apparent when the membrane was previously hyperpolarized to levels below −90 mV (see Figs. 7, B and E, and 8C). The mean steady-state discharge of these 26 neurons in response to membrane depolarization alone was 7 ± 1 spikes/s. When membrane depolarization was preceded by 2 s of membrane hyperpolarization (−94 ± 2 mV), the mean steady-state discharge dropped significantly (1 ± 0.03 spikes/s, 86% reduction in steady-state firing). This reduction in mean firing rate included 15 neurons (14 EL and 1 CL neuron) in which prior membrane hyperpolarization was sufficient to completely eliminate all steady-state discharge during the subsequent 2-s depolarization. Analysis of the intrinsic membrane properties of those neurons expressing only an initial DE versus those neurons expressing both an initial DE and steady-state slowing of discharge after hyperpolarization demonstrated no significant differences in action potential width, Rm, or RMP between groups of neurons.

To test the possible involvement of A-current in producing the DE and the prolonged slowing of the steady-state firing frequency, 4-AP (1.5–5 mM) was added to the bath of four EL neurons. In all four neurons, application of 4-AP markedly reduced or eliminated DE (see Fig. 7, A–C) and the slowing of the steady-state firing frequency (see Fig. 7, D–F). Bath application of 4-AP also produced a depolarization of the resting Vm (−59 ± 8 mV) before vs. −52 ± 1 mV during 4-AP; n = 4), decreased membrane Rm (142 ± 85 MΩ before vs. 98 ± 52 MΩ during 4-AP), and substantially increased synaptic noise (see Fig. 7F). Recovery of DE and the return of a prolonged slowing of the steady-state discharge were observed after 4-AP washout.

PIR. After the offset of hyperpolarization and the return to resting Vm, 15 LPBN neurons demonstrated a brief burst of discharge (see Fig. 8) or PIR. PIR was observed in 11 EL and 4 CL neurons. Comparisons between those neurons expressing PIR and all other neurons revealed no significant differences in action potential characteristics, Rm, RMP, or membrane time constant. Eight of fifteen neurons expressing PIR were also shown to express DE (including 7 EL neurons and 1 CL neuron). In these neurons, when membrane depolarization was immediately preceded by a low level of hyperpolarization (i.e., from −70 to −80 mV), there was often an increase in the initial peak firing frequency or PIR (see Fig. 8B). However, when the depolarizing pulse was preceded by membrane hyperpolarization to levels greater than −80 mV, the PIR was replaced by a delay in the onset of firing (DE, see Fig. 8C). All eight neurons that coexpressed PIR and DE also demonstrated a prolonged slowing of the steady-state discharge when membrane hyperpolarization preceded depolarization. Comparisons between those neurons expressing both DE and PIR versus those neurons expressing only PIR revealed a significantly higher Rm in those neurons expressing PIR only (192 ± 29 vs. 128 ± 14 MΩ, PIR only vs. PIR + DE, respectively; P < 0.04). There was, however, no significant difference in action potential characteristics, membrane time con-
stant, or RMP between neurons expressing PIR versus those expressing PIR + DE.

Electrophysiology and morphology. Eleven LPBN neurons were electrophysiologically characterized and intracellularly labeled with biocytin (see Fig. 9). Seven of these were located in the EL subnucleus, and four were located in the CL subnucleus. The majority of biocytin-filled neurons (8/11) were ovoid and had two to three primary dendrites (see Fig. 9, B-D). The long axis of these cells was 10–15 µm, and the shorter axis was 7–10 µm. The remaining three neurons had larger and more elongated cell soma, with three to five primary dendrites (see Fig. 9A). The long axis of these large fusiform neurons averaged 32 µm, and the short axis averaged 11 µm. The three larger, fusiform-shaped neurons were all found in the EL subnucleus.

DISCUSSION

The results of our study provide the first evidence for differences in the electrophysiological profiles of neurons both within and between anatomically defined subnuclear regions of the LPBN in the adult rat. In general, we found that neurons located in the CL subnucleus had significantly shorter action potential widths and less SFA adaptation during membrane depolarization compared with EL neurons. These findings cannot be explained on the basis of differences in the mean RMP, R_N, or membrane time constant of neurons located in the EL versus those located in the CL subnucleus. In addition, a large percentage of LPBN neurons, equally distributed throughout the EL and CL subnuclei, were shown to express DE, a repetitive firing property that may be important in determining their responses to synaptic drive. Contrary to our hypothesis, our limited evidence suggests that the differing properties of LPBN neurons are not linked to anatomically distinct neurons or confined to a particular subnucleus.

Several previous studies have examined the general electrophysiological properties of LPBN neurons in vitro, including action potential characteristics and resting membrane properties (6, 11, 27, 30, 32). To our knowledge, however, only two previous studies have suggested that the LPBN may contain a nonuniform group of neurons. In one study examining glutamate receptor subtypes in the LPBN, Zidichouski and colleagues (30) identified two subgroups of neurons based on action potential profiles. Post hoc analysis of AHP shape showed that approximately two-thirds of LPBN neurons exhibited slow, unnotched AHPs. These neurons tended to have a higher R_N and membrane time constant when compared with the remaining one-third, which had slow AHPs modified with a brief afterdepolarization notch. The subnuclear location of these different types of neurons however was not discussed. In a recent study by Kobashi and Bradley (23), the intrinsic membrane properties of neurons in the EL and dorsal LPBN were compared with medial parabrachial neurons in the neonatal rats. Although different regions of the LPBN were examined compared with our study, the results from this second study demonstrated significant differences in some intrinsic properties between subnuclei (R_N and membrane time constant were significantly different between the EL and dorsal LPBN subnuclei), but no significant differences were observed.
for other intrinsic properties (RMP or action potential width was not significantly different across subnuclei).

Repetitive firing characteristics. Our study is the first to describe the repetitive discharge properties of adult LPBN neurons in vitro. We found that EL neurons had more rapid SFA compared with CL neurons. However, within each subnucleus, a wide range of percent SFA was observed (70% of EL neurons expressed >80% SFA and 23% of CL neurons expressed >80% SFA). To our knowledge, only Kobashi and Bradley (23) have previously examined the repetitive firing properties of LPBN neurons in vitro. In their study, two main patterns of repetitive discharge were recorded from LPBN neurons. Some neurons demonstrated a delay in the onset of the first action potential during membrane depolarization and had low, irregular repetitive discharge rates during 1.5 s of depolarization. In contrast, other neurons had little delay in the onset of action potential discharge during depolarization and had higher repetitive discharge rates. Both types of neurons were identified to be present in the two LPBN subnuclei examined (EL vs. dorsal LPBN), and the ratio of the two types of neurons within each subnucleus was approximately equal (60% of the EL neurons and 63% of dorsal LPBN neurons were described as the more rapidly adapting neurons). A comparison of the number of action potentials evoked during 1.5 s of depolarization, however, demonstrated a trend for EL neurons to generate fewer action potentials compared with neurons in the dorsal LPBN. Although CL neurons were not recorded in their study, the results of Kobashi and Bradley’s study are remarkably similar to ours; there appears to be a trend, in both the adult and the neonatal preparation, for the neurons in the EL subnucleus to demonstrate greater SFA compared with neurons located in other LPBN subnuclei. This is despite the fact that both studies have clearly demonstrated a mixture of repetitive discharge characteristics distributed among the neurons within a single LPBN subnucleus.

Interestingly, in vivo (14, 15) studies of the repetitive firing properties of LPBN neurons have demonstrated very little SFA. These minimally adapting LPBN neurons recorded in vivo have been reported to be distributed throughout the CL and EL subnuclei, consistent with the distribution of less rapidly adapting neurons we observed in vitro. The lack of identification of a group of more rapidly adapting LPBN neurons in vivo may reflect the relative small sample size from these in vivo studies.
vivo studies. Alternatively, there may be some masking of the SFA by the interplay of intrinsic membrane properties with other major determinants of firing rate, including synaptic inputs and the presence of multiple neuromodulators in vivo. Further investigation is needed to determine whether more rapidly adapting neurons can be converted to nonadapting neurons by any of the neuromodulators identified to be present in the LPBN (4). To date, the presence of DE in LPBN neurons in vivo has not been investigated; therefore, it also remains to be identified whether the repetitive discharge of LPBN neurons can be modulated by prior membrane hyperpolarization in vivo.

Currents activated by membrane hyperpolarization. The presence of both DE and PIR, two prominent modulators of synaptic plasticity in neural circuits, has not been previously described in LPBN neurons. We found that 70–80% of neurons located in both the EL and CL subnuclei of the LPBN expressed DE, a delay in the onset of firing when depolarized from hyperpolarized $V_m$, including a subpopulation of neurons that had significantly lower RMPs and displayed a delay in discharge when depolarized from rest. The duration of delay was shown to be dependent on both the magnitude and the duration of hyperpolarizing prepulse, consistent with the removal of inactivation of a transient outward potassium current, or A-current (8, 19). In some neurons expressing DE, hyperpolarizing pulses induced a more prolonged suppression of neuronal discharge that persisted for the duration of the 2-s depolarizing pulse and often completely silenced the neuron. This longer duration suppression was also time and voltage dependent, suggesting the presence of an A-current with a longer time course of decay. The existence in other brain regions of A-currents with two different time courses of inactivation (fast and slow) has been previously described (25, 29). The application of a high dose of 4-AP diminished or completely eliminated both the transient and the more prolonged slowing of action potential responses to depolarizing current produced by a prior membrane hyperpolarization. This observation supports the notion that the inhibition of neuronal responses after hyperpolarizing currents was mediated by A-current, as has been demonstrated unequivocally by others examining this phenomenon in more detail in other central nervous system neurons (8, 25, 29). In the present study, however, direct measurements of membrane currents were not made, and so the involvement of other currents cannot be excluded.

A subgroup of LPBN neurons also demonstrated PIR, an augmented response to depolarizing current, after membrane hyperpolarization. In 8 of 11 of the neurons expressing PIR, DE could also be induced. In these neurons, small hyperpolarizing prepulses enhanced the initial action potential response to depolarization, whereas larger hyperpolarizing prepulses caused a distinct delay in the onset of spike discharge (DE) and a slowing of the steady-state firing at higher levels of hyperpolarization. Coexistence of DE and PIR in single neurons has been described in other central nuclei and has been suggested to contribute to the generation of rhythmic discharge in neurons (7, 19). Rhythmically discharging neurons in the EL subnucleus of the LPBN have been recorded in vivo and appear to be associated with inputs from the respiratory central pattern generator (10, 15). Our findings suggest that LPBN neurons may be rhythmically entrained by a combination of both intrinsic neuronal properties and strong synaptic inputs.

Anatomical considerations. In the present study, we were not able to demonstrate a correlation between cell morphology and electrophysiological properties of LPBN neurons. Although the larger, fusiform cells studied were all classified as rapidly adapting neurons and were confined to the EL subnucleus, the number of labeled cells was small. Arguing against such a correlation were the smaller ovoid neurons, which were found throughout the two subnuclei studied and which expressed the full spectrum of SFA properties. However, our morphological data are in general agreement with previous reports (13) suggesting that the larger multipolar cells are located primarily in the EL subnucleus.
Functional considerations. The LPBN is a site of convergence of afferent inputs from a number of cardiorespiratory-related brain sites, as well as a source of projection neurons that are distributed to autonomic regulatory centers both rostral and caudal to the pons (2, 13, 18, 21, 28). Although the LPBN is comprised of anatomically discrete subnuclei with well-defined connections, too little is known to attribute single physiological functions to specific LPBN subnuclei (12, 18). The results of our study, however, demonstrate that neurons with the greatest SFA are confined to the EL subnucleus, suggesting the possibility that those cells might subserve some specific function defined by the combination of location and electrophysiological profile. Our results suggest that either the type of afferent input these neurons receive does not require high-frequency encoding or the function of these neurons may be to dampen the response to excitatory afferent inputs. The EL subnucleus projects to the central nucleus of the amygdala, so that possible roles might be in integrating or triggering emotional responses to somatosensory stimuli (12, 13). In contrast, neurons demonstrating less SFA were found to be distributed throughout the CL and EL subnuclei of LPBN. This observation, combined with their limited adaptation, suggests that these neurons might function either as interneurons or as relay neurons, faithfully transmitting afferent information to adjacent subnuclei or to the next site in a central pathway. In the present study, backlabeling of potential target nuclei was not performed, so we can only speculate on the axonal trajectories of the different types of neurons identified.

In our study, the majority of LPBN neurons expressed DE, regardless of subnuclear location. Based on the presence of DE, it may be predicted that neuronal responses to excitatory inputs would be modified substantially by a preceding barrage of inhibitory postsynaptic potentials. With regard to possible sources of inhibitory inputs to LPBN neurons, we and others have demonstrated that a large number of LPBN neurons receive input from peripheral baroreceptors and that the predominant effect of baroreceptor activation on LPBN neurons is inhibitory (16, 20). Thus increases in arterial pressure and baroreceptor input, among others, may substantially modulate the timing and pattern of LPBN neuronal output through this mechanism. Alternatively, an interneuronal network may be important in modulating LPBN neuronal output. During the application of 4-AP and blockade of A-current, we observed membrane depolarization and a large increase in synaptic noise within our slice preparation, suggesting the presence of a tonically active local inhibitory circuit. The presence of an interneuronal network has been previously identified in vitro by Zidichowski and colleagues (30, 31) and has been utilized to investigate the role of both excitatory and inhibitory synaptic transmission within the LPBN. It has also been hypothesized from in vivo stimulation studies that this interneuronal network may be involved in generating cardiovascular responses evoked by stimulation of the EL subnucleus, but this remains to be determined (5).

The results of the present study suggest that the output of neurons located in the EL and CL subnuclei of LPBN could be strongly influenced by their intrinsic membrane properties. Analysis of neuronal function by subnuclear location demonstrated significant differences in the average SFA and action potential width of neurons located in the EL versus the CL subnucleus. However, the observation that a mixture of rapidly adapting and more slowly adapting neurons and of neurons with and without DE was present in both subnuclei suggests that clear differences in repetitive firing characteristics between subnuclei may not exist in the LPBN. The lack of a distinct electrophysiological “type” of neuron within a single subnucleus of the LPBN was supported by anatomical evidence demonstrating that similarly shaped neurons present in both subnuclei had diverse electrophysiological properties. Thus, although our data do not support our original hypothesis, they do provide new insights into the complex cellular properties that contribute to visceral integration at the LPBN level.

This research was supported by the American Heart Association (IA-GS-39 and 95014480; L. F. Hayward), the National Heart, Lung, and Blood Institute Grant HL-14388 (R. B. Felder), and the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (R. B. Felder).

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Received 16 March 1998; accepted in final form 6 November 1998.

REFERENCES


9. Dekin, M. S., P. A. Getting, and S. M. Johnson. In vitro characterization of neurons in the ventral part of the nucleus...


