Postnatal Development Differentially Affects Voltage-Activated Calcium Currents in Respiratory Rhythmic Versus Nonrhythmic Neurons of the Pre-Bötzinger Complex

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Elsen, Frank P. and Jan-Marino Ramirez. Postnatal development differentially affects voltage-activated calcium currents in respiratory rhythm versus nonrhythm neurons of the pre-Bötzinger complex. J Neurophysiol 94: 1423–1431, 2005. First published May 11, 2005; doi:10.1152/jn.00237.2005. The mammalian respiratory network reorganizes during early postnatal life. We characterized the postnatal developmental changes of calcium currents in neurons of the pre-Bötzinger complex (pBC), the presumed site for respiratory rhythm generation. The pBC contains not only respiratory rhythm (R) but also nonrhythm neurons (nR). Both types of neurons express low- and high-voltage-activated (LVA and HVA) calcium currents. This raises the interesting issue: do calcium currents of the two colocalized neuron types have similar developmental profiles? To address this issue, we used the whole cell patch-clamp technique to compare in transverse slices of mice LVA and HVA calcium current amplitudes of the two neuron populations (R and nR) during the first and second postnatal week (P0–P16). The amplitude of HVA currents did not significantly change during early postnatal development, suggesting that the observed amplitude changes in nR pBC-neurons are caused by (DHP) insensitive calcium currents. The ratio between HVA calcium current amplitudes dramatically changed during early postnatal development: At P0–P3, current amplitudes were significantly larger in R pBC-neurons (P0–P16), but it significantly increased in nR pBC-neurons during P8–P16. The dehydropyridine (DHP)-sensitive current amplitudes did not significantly change during the early postnatal development, suggesting that the observed amplitude changes in nR pBC-neurons are caused by (DHP) insensitive calcium currents. The ratio between LVA calcium current amplitudes dramatically changed during early postnatal development: At P0–P3, current amplitudes were significantly larger in R pBC-neurons, whereas at P8–P16, current amplitudes were significantly larger in nR pBC-neurons. Our results suggest that calcium currents in pBC neurons are differentially altered during postnatal development and that R pBC-neurons have fully expressed calcium currents early during postnatal development. This may be critical for stable respiratory rhythm generation in the underlying rhythm generating network.

I N T R O D U C T I O N

Fetal breathing movements are important for the normal development of the lungs (Harding et al. 1993; Kitterman 1996). Obviously the respiratory neuro-muscular system should be fully functional at birth to ensure the survival of the infant. However, the mammalian CNS undergoes a complex reorganization of its neuronal structures during the first postnatal week. Major changes occur in the distribution and expression of neurotransmitter receptors (Laurie et al. 1992; Zhang et al. 2002). Significant postnatal changes have been described for glycinergic and GABAergic synaptic transmission (O’Brien et al. 2004; Singer et al. 1998) as well as leak and voltage-dependent ion channels of respiratory motoneurons (Bayliss et al. 1994; Kanjhan et al. 2004; Viana et al. 1994). Different studies reported alterations of the respiratory motor pattern (Paton and Richter 1995; Smith et al. 1990). Recent studies emphasized the important role of developmental transcription to define the respiratory rhythmic pattern (Borday et al. 2004).

The respiratory motor pattern is generated in neuronal networks located within the ventrolateral medulla and includes a region, called the pre-Bötzinger complex (pBC). (Ramirez et al. 1998; Schwarzacher et al. 1995; Smith et al. 1991). Containing the pBC and the hypoglossal nucleus (XII), a brain stem slice preparation of mice (Funk et al. 1994; Ramirez and Richter 1996; Smith et al. 1991) generates rhythmic respiratory activity (Lieske et al. 2000; Pena and Ramirez 2004). Using this slice preparation, Ramirez and colleagues (Ramirez et al. 1996) demonstrated qualitatively that the generated rhythmic respiratory motor output undergoes significant modifications during postnatal development. Relatively little is known about the postnatal changes within the presumed rhythm-generating network (pBC) because the neurons located within this region are heterogeneous: the pBC contains respiratory rhythm neurons but also neurons that are apparently unrelated to neuronal control of breathing. It has been discussed that pBC neurons like the preinspiratory neurons might be involved in fictive swallowing and vomiting (Zheng et al. 1997). Moreover there is great functional overlap among respiratory, cardiovascular, and thermoregulatory control within the ventrolateral medulla, which presumably also includes areas within the pBC (Dick and Morris 2004; Nakamura et al. 2005).

It is assumed that the respiratory neurons in the pBC form excitatory and inhibitory synaptic interactions between each other and have specific membrane properties that are critical for generating and shaping the respiratory rhythm (Ballanyi et al. 1992; Del Negro et al. 2002; Funk and Feldman 1995; Pena and Ramirez 2004; Ramirez and Richter 1996; Ramirez et al. 1997; Thoby-Brisson and Ramirez 2001). In vivo studies demonstrated the involvement of low- and high-voltage-activated calcium currents (LVA and HVA) in postsynaptic events of the membrane potential (Champagnat and Richter 1994; Pierrefiche et al. 1995). Furthermore, in vitro studies could qualitatively (Onimaru et al. 1996) and quantitatively (Elsen and Ramirez 1998) show that respiratory neurons express calcium currents that may contribute to the generation of the respiratory rhythm.

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In this study, we used transverse brain stem slice preparations to compare the postnatal development of voltage-activated calcium currents between respiratory rhythmic and nonrhythmic neurons that co-exist in the pBC. We investigated specifically the developmental changes of LVA and HVA currents in both groups of pBC neurons to determine whether rhythmic and nonrhythmic neurons have different developmental profiles.

METHODS

Preparation and solutions

All experiments were performed in the transverse medullary slice preparation (Fig. 1A) obtained from 0- to 16-day-old neonatal mice (CD1 mice). The mice were deeply anesthetized with ether and decapitated at the C3/C4 spinal level. All steps to obtain functional slice preparations have been published elsewhere (Ramirez et al. 1996) and shall only briefly be summarized in this study. The brain stem was isolated in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 128 NaCl, 3 KCl, 1.5 CaCl2, 1 MgSO4, 24 NaHCO3, 0.5 NaH2PO4, and 30 d-glucose equilibrated with carbogen (95% O2-5% CO2; pH 7.4).

Secured in a vibratome with the rostral end up, thin slices were sectioned serially from rostral to caudal until reaching the rostral boundary of the pBC. We recognized this region by cytoarchitectonic landmarks, such as inferior olive (IO), nucleus of the solitary tract (NTS), hypoglossal nucleus (XII), and nucleus ambiguous (NA), and the absence of the facial nucleus (Fig. 1A). A 550- to 650-μm-thick section was made caudal of this rostral boundary, and the resulting rhythmic slice was immediately transferred into a recording chamber. The preparation was submerged with its rostral surface up under a stream of ACSF (temperature, 29°C; flow rate, 16 ml/min) and stabilized for 10 min. As it is common in the field of respiration, the recording solution containing (in mM) 110 CsCl, 30 TEA-Cl, 1 CaCl2, 10 EGTA, 2 MgCl2, 4 Na2ATP, and 10 HEPES (pH 7.2).

The preparation was submerged with unpolished patch electrodes. These electrodes were manufactured from borosilicate glass pipettes with filament (Warner Instruments G150F-4). The electrodes had a resistance of 3–5 MΩ when filled with the whole cell patch-clamp pipette solution containing (in mM) 110 CsCl, 30 TEA-Cl, 1 CaCl2, 10 EGTA, 2 MgCl2, 4 Na2ATP, and 10 HEPES (pH 7.2).

The patch-clamp experiments were performed with a patch clamp amplifier (AxoPatch 1D), a digitizing interface (Digidata 1200A), and the software programs pClamp 6.3 and 8.0 (Axon Instruments). The data were analyzed off-line with the software programs ClampFit 6.3 and 8.0 (Axon Instruments) and further statistical analysis was performed with the software program Prism 3.0 (Graphpad).

All quantitative data are given as means ± SE, if not indicated otherwise. Significance was assessed with the Student’s t-test, one- or two-way ANOVA and significance was assumed for values P < 0.05.

Neurons located at least three to four cell layers (~80–150 μm) caudal from the rostral surface of the slice were recorded under visual control. Neurons located directly at the slice surface were not examined because they were more likely damaged during the preparation stages. Neurons located deeper within the slice were identified as rhythmic (R) if the intracellularly recorded rhythmic activity was in phase with the population activity recorded from the contralateral pBC (Fig. 1C; for details, see: Ramirez et al. 1996). Important note: we could not discriminate between different types of respiratory neurons because of the specific recording conditions that are necessary to isolate calcium currents (see next paragraphs for more detailed explanation).

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mic” neurons (nR; Fig. 1D). After discriminating R from nR neurons in the functional network, TTX was applied into the bath; this blocked network activity irreversibly. Thus it was not possible to record from more than one neuron in each slice preparation.

We compared the postnatal development of electrophysiological properties of R (n = 76) and nR (n = 212) pBC neurons during the first 16 days of postnatal development. At older stages of postnatal development (P12–P16), it became increasingly difficult to obtain useful whole cell patch-clamp recordings because of the increasing density of tissue myelination. Initially we divided our data into two groups: the first postnatal week (P0–P7) and the second postnatal week and older (P8–P16). To detect possible changes during the early perinatal stages of development, we further divided the first postnatal week into two equal groups (P0–P3, P4–P7), creating a total of three age groups for our investigations (P0–P3, P4–P7, P8–P16).

The characterization of calcium currents required a complete block of potassium currents. This was best achieved with an intracellular pipette solution containing CsCl and TEA. However, this pharmacological isolation alters the discharge characteristics of respiratory neurons. For this reason, we were unable to differentiate between different types of respiratory neurons. Thus throughout this study neurons were only classified as either respiratory or nonrespiratory neurons. This discrimination was unaffected by the intracellular recording solution.

Current response traces were recorded with either off- or on-line leak subtraction (P/4 protocol), eliminating the linear leak current and residual capacity currents. The liquid junction potential was 2 mV and was manually subtracted with the amplifier’s pipette offset regulator before the acquisition was started.

We have to emphasize that whole cell voltage-clamp recordings from neurons embedded in a functional network are accompanied by difficult clamp control. This could lead to incorrect values for current amplitudes. Thus recordings with obvious space clamp problems (Armstrong and Gilly 1992; White et al. 1995) were discarded. Poor space clamping was indicated by rebound spikes (rapid, fast inactivating inward currents, which were induced by steps from depolarizing test potentials to the former holding potential) or an increase in the delay to onset of an inward current with increasing magnitude of test pulse. Steps to higher test potentials were typically associated with a reduction in delay to current onset. We also discarded neurons with insufficiently blocked K+ currents. This was evident in outward currents typically commencing at voltage steps to 10 mV.

Note that the number of neurons that were quantitatively evaluated is not always consistent with the number of qualitative observations.

In our computer analyses, we were able to obtain average data only from those neurons that were examined with identical experimental protocols. Most recordings were therefore evaluated quantitatively for only a limited number of aspects. All substances used in this study were obtained from SIGMA, except TTX, which was obtained from Alomone Labs.

Pharmacological isolation of whole cell voltage-activated calcium currents

Using the conventional patch-clamp technique, voltage-activated calcium currents were pharmacologically isolated by intracellular blockade of voltage-activated potassium currents with 110 mM CsCl and 30 mM tetraethylammonium (TEA) chloride. As shown in a previous study (Elsen and Ramirez 1998), this blockade was sufficient for the investigation of the maximal inward calcium current amplitude. In addition, voltage-activated inward sodium currents were blocked by extra cellular bath application of 0.5 μM tetrodotoxin (TTX). The remaining currents appeared to be voltage-activated calcium currents, since they were blocked by bath application of 200 μM cadmium chloride (example shown in Fig. 5, A and B).

RESULTS

Changes of passive properties of R and nR neurons during the postnatal development

Using the relationship of voltage, current, and resistance given by Ohm’s law, the input resistance was calculated from the difference of the current amplitudes evoked by a 20-mV test step from a holding potential of −60 to −80 mV. The capacitance of each recorded neuron was obtained from the capacitance unit of the amplifier after 80% compensation and before the acquisition was started.

R pBC neurons. Input resistance and capacitance values were not significantly different when compared between the three age groups (P0–P3, P4–P7, P8–P16; 1-way ANOVA: P > 0.05; Table 1). More specifically, the input resistance at age P8–P16 decreased significantly compared with the age group of P0–P3. The linear decrease of the input resistance from age P0–P3 to P8–P16 was significant (linear trend: P = 0.05). Capacitance values for the three age groups were not significantly different.

To compare the values of the different age groups of R and nR pBC neurons with each other, we used a two-way ANOVA significance test. The input resistance values during the investigated development were not significantly different between R and nR pBC neurons. The capacitance did not significantly differ between age group P0–P3 and P8–P16 (*, P = 0.0053).

TABLE 1. Electrophysiological properties of different age groups from rhythmic and non-rhythmic pBC neurons

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Age Groups</th>
<th>HVA: Amplitude, pA</th>
<th>LVA: Amplitude, pA</th>
<th>R, MΩ</th>
<th>Capacitance, pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhythmic</td>
<td>P0–P3</td>
<td>−579 ± 87.0 (33)</td>
<td>−900 ± 381.3 (3)</td>
<td>273 ± 37.7</td>
<td>18.7 ± 2.0</td>
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<tr>
<td></td>
<td>P4–P7</td>
<td>−574 ± 81.3 (27)</td>
<td>−538 ± 192.8 (4)</td>
<td>283 ± 31.0</td>
<td>14.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>P8–P16</td>
<td>−609 ± 115.9 (16)</td>
<td>−303 (2)</td>
<td>239 ± 52.6</td>
<td>14.5 ± 2.8</td>
</tr>
<tr>
<td>Nonrhythmic</td>
<td>P0–P3</td>
<td>−383 ± 36.1 (95)</td>
<td>−653 ± 166.9 (6)</td>
<td>370 ± 23.4</td>
<td>15.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>P4–P7</td>
<td>−598 ± 57.5 (57)</td>
<td>−460 ± 208.0 (4)</td>
<td>345 ± 26.4</td>
<td>14.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>P8–P16</td>
<td>−1077 ± 112.6 (60)*</td>
<td>−368 ± 81.3 (7)</td>
<td>247 ± 36.0*</td>
<td>16.6 ± 1.6</td>
</tr>
</tbody>
</table>

In rhythmic neurons (R), a one-way ANOVA test revealed that the low- and high-voltage-activated (LVA and HVA) amplitudes did not significantly change during the observed developmental ages. In non-rhythmic neurons (nR), the LVA amplitudes did not significantly change, but the HVA amplitudes did significantly increase (*, P < 0.0001) and a linear trend was detected, although no significance was found between R and nR neurons 2-way ANOVA: P > 0.05). The input resistance (R) values were determined by a voltage step from −60 to −80 mV. In nR neurons, a one-way ANOVA test revealed a significant decrease R between age group P0–P3 and P8–P16 (*, P = 0.0053). The values for the cell capacitance were read off the amplifier adjustment controls after 80% series resistance compensation and were not significantly different from each other. All values are given as means ± SE, and the numbers in the parentheses refer to the respective number of experiments.
Developmental changes of the HVA calcium current amplitude in R and nR neurons

Using the conventional patch-clamp technique (Hamill et al. 1981), whole cell HVA calcium currents were evoked from a holding potential ($V_h$) of −60 mV to different test potentials ($V_t$) from −80 to 20 mV. A current trace example is shown in Fig. 2A. To quantify the relation between current amplitude and test potential, the measured peak current values were plotted against the respective test potentials (Fig. 2B, I-V curve). Average I-V curves for all three age groups are shown in Fig. 2, C (R) and D (nR). The maximum current amplitude was between a peak voltage potential of −20 to −10 mV for R and nR pBC neurons throughout all three age groups. To characterize the developmental change of the maximum current amplitude, the current value at the peak voltage was determined by a single voltage step from −60 to −10 mV. Example current traces for each age group are shown in Fig. 3, A (R) and B (nR).

RHYTHMIC pBC-NEURONS. The mean current amplitudes are shown as a bar diagram in Fig. 3C (■). Absolute mean amplitude values for each age group are given in Table 1. Throughout the investigated development no significant difference among the three age groups ($P \geq 0.05$) could be detected (1-way ANOVA). The absence of a significant difference ($P \geq 0.05$) was also noted when the investigated neurons were initially divided into only two groups: first postnatal week (P0–P3) and second postnatal week and older (P8–P16; data not shown).

NR pBC NEURONS. The mean current amplitudes are shown as a bar diagram in Fig. 3C (○). Table 1 contains the mean amplitude values for each age group. In contrast to the R pBC neurons, a one-way ANOVA test for the nR pBC neurons revealed a highly significant difference between the age groups ($P \leq 0.0001$), and a highly significant linear trend ($P < 0.0001$) was determined toward larger amplitudes in older animals. More specifically, the current amplitude at age P8–P16 increased significantly ($P \leq 0.001$, Fig. 3C, *) compared with the age groups of P0–P3 and P4–P7. A significant increase was also noted ($P \leq 0.001$) when the data were grouped into only two groups: first (P0–P7) and second postnatal week and older (P8–P16; data not shown).

Using a two-way ANOVA test, we compared the three age groups of R and nR pBC neurons with each other. A highly significant difference ($P \leq 0.001$) among the age groups was detected, but no significant difference ($P \geq 0.05$) between R and nR pBC neurons could be found.

Comparison of HVA I-V curves in R and nR neurons between age groups P0–P3 and P8–P16

At age group P0–P3, the R pBC neurons did express a significantly ($t$-test: $P \leq 0.05$) larger current amplitude at the peak voltage ($V_t = -10$ mV) than nR pBC neurons (Fig. 4A). However, at the older age group P8–P16, we observed the opposite result; here the R pBC neurons expressed a significantly ($P \leq 0.05$) smaller current amplitude compared with the nR pBC neurons (Fig. 4B). Considering the results reported in the previous paragraph, this difference was due to the significant increase in the current amplitude of nR pBC-neurons at age group P8–P16, whereas the current amplitudes in R pBC neurons remained relatively stable and did not significantly change during the investigated ages.

Pharmacological data

As shown in an earlier study (Elsen and Ramirez 1998), pBC neurons express dihydropyridine-sensitive and -insensitive
HVA calcium currents. To discriminate between these two classes of calcium current subtypes, we applied 4 μM nifedipine into the bath solution. To observe the pharmacological blockade of the maximum current amplitude, the current value at the peak voltage was determined by a single voltage step from −60 to −10 mV. The remaining dihydropyridine-insensitive calcium currents were completely blocked by bath application of 200 μM cadmium chloride (Fig. 5A). To demonstrate the time course of the pharmacological effects, we applied a single voltage step every 10 s and plotted the current amplitude values against the elapsed time (Fig. 5B). To quantify the percentage of expressed dihydropyridine-sensitive HVA calcium current (L-type calcium current) (Fox et al. 1987), we normalized the amount of blocked current amplitude after nifedipine application to the respective control current amplitude before nifedipine application (Fig. 5B). The expressed mean percentage part of L-type calcium currents are shown as a bar diagram for each age group in Fig. 5C. Bath application of 4 μM nifedipine blocked 29.1 ± 8.47% of the current amplitude in age group P0–P3 (n = 6), 26.0 ± 4.80%
in age group P4–P7 (n = 12), and 37.5 ± 8.91% (n = 6) in age group P8–P16. No significant difference (P ≤ 0.05) was detected among the different age groups (1-way ANOVA).

Developmental changes in LVA calcium current amplitudes in R and nR neurons

LVA calcium currents were isolated from the HVA calcium currents with an off-line subtraction protocol (Bean 1985). From a V_h of -90 mV, both LVA and HVA components were evoked by depolarizing voltage steps, whereas mainly the HVA current was activated when using a V_h of -60 mV (Elsen and Ramirez 1998). To isolate the LVA calcium current from the HVA currents, we subtracted the current traces representing only HVA currents (V_h = -60 mV) from the current traces containing both components (V_h = -90 mV). An example for an isolated LVA calcium current trace is shown in Fig. 6A. The corresponding I-V curve is shown in Fig. 6B. The peak voltage for the LVA calcium currents was between -40 and -50 mV throughout the investigated ages for R and nR pBC neurons. To quantify the developmental changes of the maximal LVA calcium current amplitude, we measured the current amplitude at the peak voltage (Fig. 6C).

R pBC neurons. The mean current amplitudes are shown in Fig. 6C and the mean current amplitude values are reported in Table 1. A one-way ANOVA test revealed no significant differences among the age groups (P ≥ 0.05).

nR pBC neurons. The mean current amplitudes are shown as a bar diagram in Fig. 6D, and Table 1 contains the absolute mean amplitude values for each age group. Similar to the R pBC neurons, a one-way ANOVA test for the nR pBC neurons revealed no significant difference among the age groups (P ≥ 0.05).

Using a two-way ANOVA test, we compared the age groups of R and nR pBC neurons with each other. No significant difference between the age groups or between R and nR pBC neurons was detected.

Discussion

It has been demonstrated that neurons of the respiratory network express LVA and HVA calcium currents (Elsen and Ramirez 1998; Onimaru et al. 1996). Most computational models of respiratory rhythm generation include the contribution of voltage-activated calcium currents in the rhythm-generating process (Ramirez and Richter 1996). We characterized the developmental changes of calcium currents in neurons of the pBC, the presumed kernel for respiratory rhythm generation (Lieske et al. 2000; Smith et al. 1991). Our results show that the amplitude of HVA calcium currents remained stable in respiratory rhythmic pBC neurons throughout the investigated postnatal development (P0–P16). As suggested in some computer models, the high expression of developmental stable calcium current amplitudes could provide the necessary depolarization to ensure the rhythm generation in the pBC right after birth and throughout the early postnatal development. In contrast to the observed stability in respiratory pBC neurons, we found that in nR pBC neurons the amplitude of HVA calcium currents was significantly smaller, but then it increased significantly after the first postnatal week. This developmental increase of HVA currents is well documented for different cell types, including retinal ganglion cells (Schmid and Guenther 1999), spinal motor neurons (Gao and Ziskind-Conhaim 1998; Jiang et al. 1999), and pyramidal neurons (Lorenzon and Foehring 1995). However, in hypoglossal motoneurons HVA calcium current densities remained unchanged during development (Miles et al. 2004). It is known that not all neurons in the
pBC are involved in basic respiratory rhythm generation. The nR pBC neurons that we observed could be inactive during regular in vivo breathing and might be recruited during more severe respiratory conditions, or they might be involved in completely other functions such as vomiting, swallowing, or vocalization (Dick and Morris 2004; Nakamura et al. 2005; Zheng et al. 1997). Therefore they would not necessarily need the same high calcium current expression as observed in the R pBC neurons.

The amplitude of the LVA calcium current tended to decrease in R and nR pBC neurons during postnatal development (P0–P16), but our data did not reach statistical significance. Although not previously studied in the pBC, there have been previous reports that suggest a reduction in LVA in hypoglossal motorneurons (Viana et al. 1993), in pyramidal neurons from the visual cortex (Tarasenko et al. 1998), in ventricular neurons (Ferron et al. 2002), and in retinal neurons (Rothe et al. 1999). However, there have also been reports of a developmental increase in LVA calcium currents, e.g., in hippocampal pyramidal neurons (Kortekaas and Wadman 1997) and in muscle cells (Beam and Knudson 1988), suggesting that also the LVA calcium current is differentially altered during postnatal development.

**Developmental changes of passive membrane properties**

It is conceivable that the developmental increase of HVA calcium current amplitudes as observed in the present study might originate from developmental morphological changes of pBC neurons. It is for example known that changes of membrane resting potential, cell capacitance, or input resistance can be correlated directly to developmental morphological changes of the investigated neurons (Hilaire and Duron 1999; Onimaru and Homma 2002). Therefore it was essential to characterize not only the developmental changes of the calcium currents but also potential changes in passive membrane properties, such as capacitance and input resistance. This allows us to exclude the influence of morphological changes on the observed electrophysiological changes.

We demonstrated that the capacitance of the pBC neurons did not significantly change, neither in R (P = 0.4637) nor in nR pBC neurons (P = 0.6350). These data suggest that the cell morphologies of the pBC neurons did not significantly change during the first and second postnatal week (P0–P16).

The input resistance of R pBC neurons did not change significantly (P = 0.7918) throughout postnatal development, but we found a significant (P = 0.0195) decrease in input resistance of nR pBC neurons after the first postnatal week (P0–P3, P4–P7). Similar developmental decrease of input resistance has been described for respiratory motoneurons (Bonansco et al. 2004; Cameron and Nunez-Abades 2000). The decrease in input resistance appears to be independent of a change in cell capacitance and may thus be due to a proliferation of ion channels and synaptic inputs.

**Functional considerations**

In this study, we observed a developmental increase of HVA calcium currents in nR pBC neurons. This is consistent with findings by Zhang and colleagues (Zhang et al. 1999). These authors described a similar increase of HVA calcium currents in pBC neurons. However, in their study inspiratory (R) and
unidentified (nR) pBC neurons were pooled together. Therefore in contrast to the present study, their results did not specifically distinguish between R and nR pBC neurons. This, however, is critical, because the developmental profile of HVA calcium currents differed significantly between both groups of pBC neurons. The ratio of HVA calcium current amplitudes between R and nR pBC neurons underwent a significant developmental change. During the first period (P0–P3) of the postnatal development, R pBC neurons expressed significantly larger amplitudes at the peak voltage (−10 mV) compared with the nR pBC neurons. This ratio changed dramatically during the second postnatal week (P8–P16) toward significantly larger amplitudes in nR pBC neurons. This change of ratio was due to a significant increase of the HVA calcium current amplitudes in nR pBC neurons coinciding with unchanged amplitudes in R pBC neurons. We speculate that higher calcium current conductance in R pBC neurons might be important for a functional respiratory network during the early postnatal development. Moreover, different studies have linked a developmental increase of HVA calcium currents to proper synaptogenesis during early development (Chambard et al. 1999; Isomura and Kato 1999; Mynlieff and Beam 1992). The relatively high expression of HVA calcium currents in R pBC neurons might reflect a similar importance for neuronal growth and development of synaptic connections of the respiratory rhythm-generating network during the early postnatal development.

Most models for respiratory rhythm generation (Koshiya and Smith 1999; Ramirez and Richter 1996; Rekling and Feldman 1998; Rybak et al. 1997a,b; Smith et al. 2000) consider the importance of voltage-activated calcium currents, thus an early developmental expression of those currents in respiratory R pBC neurons may be critical to insuring the generation of respiratory rhythmic activity. We have recently demonstrated that one type of respiratory pacemaker neurons, the cadmium-sensitive pacemaker neurons depend on the activation of calcium currents (Pena and Ramirez 2004; Ramirez et al. 2004; Thoby-Brisson and Ramirez 2001). These neurons appear to contribute to respiratory rhythm generation by activating the calcium-activated nonselective cation current (Pena and Ramirez 2004). Given that calcium currents are generally involved in the generation of action potentials (Hallworth et al. 2003; Marcotti et al. 2003), their stable postnatal expression will also be an important prerequisite for rhythm generation in network-based models of respiratory rhythm generation (Del Negro et al. 2002; Rybak et al. 1997b).

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