Current and Voltage Clamp Studies of the Spike Medium Afterhyperpolarization of Hypoglossal Motoneurons in a Rat Brain Stem Slice Preparation

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LAPE, REMIGIUS and ANDREA NISTRI. Current and voltage clamp studies of the spike medium afterhyperpolarization of hypoglossal motoneurons in a rat brain stem slice preparation. J. Neurophysiol. 83: 2987–2995, 2000. Whole-cell patch clamp recordings were performed on hypoglossal motoneurons (HMs) in a brain stem slice preparation from the neonatal rat. The medium afterhyperpolarization (mAHP) was the only afterpotential always present after single or multiple spikes, making it suitable for studying its role in firing behavior. At resting membrane potential (−68.8 ± 0.7 mV), mAHP (23 ± 2 ms rise-time and 150 ± 10 ms decay) had 9.5 ± 0.7 mV amplitude, was suppressed in Ca^{2+}-free medium or by 100 nM apamin, and reversed at −94 mV membrane potential. These observations suggest that mAHP was due to activation of Ca^{2+}-dependent, SK-type K+ channels. Carbachol (10–100 μM) reversibly and dose dependently blocked the mAHP and depolarized HMs (both effects prevented by 10 μM atropine). Similar mAHP block was produced by muscarine (50 μM). In control solution a constant current pulse (1 s) induced HM repetitive firing with small spike frequency adaptation. When the mAHP was blocked by apamin, the same current pulse evoked much higher frequency firing with strong spike frequency adaptation. Carbachol also elicited faster firing and adapting behavior. Voltage clamp experiments demonstrated a slowly deactivating, apamin-sensitive K+ current (I_{AHP}) which could account for the mAHP. I_{AHP} reversed at −94 mV membrane potential, was activated by depolarization as short as 1 ms, decayed with a time constant of 154 ± 9 ms at −50 mV, and was also blocked by 50 μM carbachol. These data suggest that mAHP had an important role in controlling firing behavior as clearly demonstrated after its pharmacological block and was potently modulated by muscarinic receptor activity.

INTRODUCTION

Hypoglossal motoneurons (HMs) innervate tongue muscles (Lowe 1980) and thus are involved in several functions like respiration, mastication, swallowing, and suckling. Indeed, considerable interest is centered on these motoneurons because their dysfunction may result in diseases like obstructive sleep apnea or sudden infant death syndrome (Gauda et al. 1987; Wiegand et al. 1991; Willinger 1989).

Studies that have used rodent brain stem slices as models to understand the basic properties of HMs have demonstrated that these cells possess several voltage-activated membrane conductances (Haddad et al. 1990; Mosfeldt Laursen and Rekling 1989; Viana et al. 1993a,b). HMs display characteristic firing patterns following membrane depolarization (Sawczuk et al. 1995, 1997; Viana et al. 1995), an aspect of special relevance because it indicates how integration of the electrical behavior at somatic HM level is eventually translated into output signals to the tongue muscles.

In response to current step injection adult HMs fire initially at high-frequency with a multicomponent decay to a much lower discharge rate (Sawczuk et al. 1995, 1997). Conversely, the majority of neonatal HMs exhibits a steady pattern of repetitive firing which is reached after a single, fast period (about 200 ms) of frequency adaptation. A smaller subgroup actually shows rapid acceleration of firing to steady state level (Viana et al. 1993b, 1995). As in the case of spinal (Barrett et al. 1980; Krnjevic et al. 1979) or facial (Nishimura et al. 1989) motoneurons, a Ca^{2+}-dependent afterhyperpolarization of medium duration (mAHP) is proposed to control adaptation of neonatal (Viana et al. 1995) and adult (Powers et al. 1999) HMs. Such an mAHP is modulated by transmitters like serotonin (5-HT) or norepinephrine (Bayliss et al. 1995; Parkis et al. 1995), raising the possibility that firing behavior is a dynamic property susceptible to changes induced by locally released transmitters. Notwithstanding the progress made by these studies, full understanding of the mechanisms underlying different degrees of repetitive firing and adaptation is still lacking.

We have attempted to reconstruct the firing behavior of neonatal HMs by starting with a quantitative description of their voltage-dependent K+ conductances to be used for computer-based modeling of action potential discharges. For this purpose we recently characterized two relatively fast, Ca^{2+}- and apamin-sensitive K+ currents (Lape and Nistri 1999). The main goals of the present study were to investigate the kinetic properties of the current underlying the mAHP, to assess its contribution to firing behavior by using the selective blocker apamin, and to ascertain the HM firing properties recorded under patch clamp conditions, as previous work had relied on sharp electrode recording (Viana et al. 1993b, 1995). Furthermore, as muscarinic receptor activity is known to affect repetitive firing probably by modulating the afterhyperpolarization (AHP) of cortical (Schwindt et al. 1988) or hippocampal (Storm 1989) neurons, we tested if this phenomenon is also applicable to HMs that apparently possess high levels of muscarinic binding sites (Rotter et al. 1979; Walmsley et al. 1981). It is presently unclear if there are functional postsynaptic muscarinic receptors on HMs as in this area there is only a report of presynaptic muscarinic action in depressing transmitter release (Bellingham and Berger 1996). A preliminary de-
scription of our work has recently appeared in abstract form (Lape et al. 1999).

**METHODS**

**Slice preparation**

Experiments were carried out using brain stem slices obtained from 0 to 6 day old rats. Thin slices were prepared following the procedure described earlier by Viana et al. (1994) and Lape and Nistri (1999). The brain stem was isolated from neonatal rats and placed into modified ice-cold Krebs solution (see **Solutions**). A tissue block containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla was then fixed (with insect pins) onto an agar block inside a Vibratome chamber filled with ice-cold Krebs containing the lower medulla.

**Recording**

Brain stem slices placed in a small recording chamber were viewed with an infrared video camera to identify single hypoglossal motoneurons within the XII nucleus. The conventional whole-cell patch-clamp recording technique (Hamill et al. 1981) was employed by using either an EPC-7 patch-clamp amplifier [for voltage-clamp (VC) experiments] or an Axoclamp-2B amplifier [for current clamp (CC) experiments]. For VC experiments patch electrodes had 3–5 MΩ DC resistance, whereas those pulled for CC patch experiments had 10–18 MΩ. Seal resistance was usually higher than 2 GΩ. After seal-rupture series resistance, Rs (5–15 MΩ), was routinely monitored and compensated (usually by 40%, range 20–60%) in VC experiments. The VC recordings were performed only when Rs stabilized and the cells were chosen for analysis if changes in Rs did not exceed 10%. The bridge was balanced routinely in CC experiments. Voltage and current pulse generation and data acquisition were performed with a PC running pClamp 6.1 software. Currents elicited by voltage steps were filtered at 3 kHz and sampled at 5–10 kHz.

**Solutions**

The Krebs solution for slice preparation and maintenance was as follows (mM): 130 NaCl, 3 KCl, 26 NaHCO3, 1.5 Na2HPO4, 1 CaCl2, 5 MgCl2, 10 glucose, 0.0004 l-ascorbic acid (290–310 mOsm). Extracellular solution for electrophysiological recording was as follows (mM): 140 NaCl, 3 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose (pH 7.4, 290–310 mOsm). Patch pipette solution was as follows (mM): 110 K-methyl-SO4, 20 KCl, 10 NaCl, 2 MgCl2, 10 HEPES, 0.5 EGTA, 2 ATP-Mg (pH 7.2, 260–270 mOsm). To prepare Ca-free Co solution CaCl2 was completely substituted with CoCl2. In various experiments muscarine chloride (muscarine), carbamylcholine chloride (carbachol), atropine methyl nitrate, and apamin were applied via the bathing solution (continuously superfused at 2–5 ml/min). Drugs were added by switching to an appropriate extracellular solution which was applied for 5–10 min for equilibration.

**Analysis**

Cell input resistance (Rin) was calculated from small (5 mV or 10 pA) hyperpolarizing voltage or current commands or from the linear portion of the I-V line (ramp test) near the cell resting potential (Vrest). To quantify the spike AHP we measured its peak amplitude (from baseline), area, time constants for monoexponential rise and decay. As these measurements are largely influenced by membrane potential, the cell resting potential was kept at the same level by intracellular current injection throughout the recording session. To measure the amplitude and decay time constants of tail currents, these were fitted with a mono- or biexponential function. The initial 5-ms record after the end of the voltage step was discarded from fitting to avoid contamination by uncompensated capacitance transients. Tail current amplitude was then obtained by extrapolating fitted curves back to the end of the voltage step. Sigma Plot and Clampfit softwares were used for exponential fitting of membrane currents and for linear regression analysis of experimental data. Data are presented as mean ± SE. All potential values were corrected off-line for the liquid junction potential, which was measured as 10 mV. Current leak subtraction was performed by using the Clampfit module. Data were analyzed statistically by using Student’s t-test or analysis of variance (ANOVA) test. Significance was accepted when P < 0.05.

**RESULTS**

Some general characteristics of the action potential (AP) and its afterpotentials elicited by intracellular current injection are presented in Fig. 1. Figure 1A shows that, in analogy with the report by Viana et al. (1993b), the decay phase of a single AP [typically evoked by a short (5 ms) current pulse; 0.5 nA]
comprised a fAHP (fast afterhyperpolarization) and a fast afterdepolarization (fADP). These early afterpotentials were followed by an mAHP which undershot the baseline for about 250 ms (Fig. 1B, same cell). An analogous mAHP (about 300-ms long) was also observed at the end of a spike train induced by a 2-s current pulse (200 pA; see Fig. 1C, different cell from A–B). In a few cells only (9% of total recorded cell number) a slow afterhyperpolarization (sAHP) (lasting 2–5 s) appeared after the mAHP (Fig. 1D; see also Viana et al. 1993b). In a larger group of cells (~40%) the mAHP was followed by a slow afterdepolarization (sADP; 1–3-s long; Fig. 1E). Because the mAHP was the most consistently observed afterpotential (present in all cells recorded; n = 59) and is known to be the target for transmitter modulation (see introduction), the present report is mainly concerned with a characterization of the mAHP.

Basic properties of mAHP

At -68.8 ± 0.7 mV resting membrane potential (V\text{rest}; n = 59 cells) the mAHP following a single AP reached peak amplitude of 9.5 ± 0.7 mV (measured from V\text{rest}) with a monoexponential rise time constant (23 ± 2 ms) from which it decayed monoexponentially (decay time constant = 150 ± 10 ms). When extracellular Ca\textsuperscript{2+} was replaced by the same concentration of Co\textsuperscript{2+}, the mAHP was abolished (Fig. 2A) but it recovered when standard external solution was reapplied (n = 4). Similar results were obtained by application (via the patch electrode) of bis-(O-aminophenoxo)-N,N',N'-tetraacetic acid (BAPTA), a selective chelator of Ca\textsuperscript{2+}, to block the action of intracellular Ca\textsuperscript{2+} raised through trans-membrane influx or release from cytoplasmic stores. In fact, out of six cells recorded with a patch electrode filled with 1 mM BAPTA and held at -68 mV, four showed no mAHP and two showed a rather small mAHP (3 and 1.5 mV, respectively).

The example of Fig. 2B indicates that, under standard recording conditions, the mAHP decreased with membrane hyperpolarization until it disappeared at -94 mV membrane potential. The inset to Fig. 2B is the plot (for 2 HMs) of the relation between membrane potential and mAHP amplitude, yielding a reversal potential of -94 mV, which coincides with the calculated equilibrium potential for K\textsuperscript{+} on the basis of the Nernst equation. This result suggests that the mAHP is a response due to increased permeability to K\textsuperscript{+}. As the mAHP was fully and irreversibly blocked by 100 nM apamin without concomitant suppression of the fAHP (Fig. 2C; similar data were observed on 9 cells), it seems likely that the K\textsuperscript{+} conductance responsible for generating the mAHP was mediated by SK Ca\textsuperscript{2+}-activated channels (Sah 1996).

Modulation of mAHP by activation of muscarinic ACh receptors

The cholinergic agonist carbachol largely attenuated the mAHP as exemplified in Fig. 3A. On average 50 \mu M carbachol reduced the mAHP to 37 ± 4% of control (n = 17 cells; P < 0.001). The action of carbachol developed quite slowly, taking about 5 min to manifest fully, was completely reversible on washout, and was dose dependent. In fact, 10 \mu M carbachol decrease the mAHP to 76 ± 5% in four cells, whereas 100 \mu M concentration reduced it to 20 ± 10% in two cells. Note that carbachol depressed the peak amplitude of the mAHP without affecting its rise or decay time course (changes in t\text{rise} and t\text{decay} were 90 ± 10% and 81 ± 9%, respectively; n = 17 cells). The action by carbachol was not accompanied by any significant change in spike amplitude (90 ± 2%), duration (107 ± 6%), or threshold (99 ± 6%).

Together with the depression of the mAHP carbachol (10–100 \mu M) also produced a dose-dependent, slowly developing and reversible depolarization of all HMs tested. For example, a 50-\mu M concentration induced a mean depolarization of 12 ± 1 mV without significant change (96 ± 4%) in R\text{in} (n = 17 cells). The carbachol depolarization persisted in the presence of TTX, thus suggesting that it had a direct action on HMs (11 ± 2 mV; n = 3).

The carbachol inhibitory action on the mAHP was prevented by 10–15 min pretreatment with 10 \mu M atropine (n = 7 cells), a selective antagonist on muscarinic ACh receptors (Fig. 3B). The mAHP however retained its sensitivity to apamin (100 nM; Fig. 3B), indicating distinctive modes of action for carbachol and this toxin in inhibiting the mAHP. Note that on average atropine per se increased the mAHP by 29 ± 7% (n = 7; P < 0.05). The selective muscarinic receptor agonist muscarine (50 \mu M) also produced HM depolarization (10 ± 4 mV).
and late action potentials in the train (see Fig. 4B). Effects similar to those of apamin were also observed by applying Ca^{2+}-free Co^{2+} solution (n = 4; not shown). Furthermore, in apamin solution firing adaptation was manifested as indicated by the continuous decline in firing frequency over time (compare time course of filled and open circle graphs in Fig. 4B). The relation between spike discharge frequency and injected current (f-I relation) was further investigated to determine the frequency coding properties of these cells. Repetitive AP discharges were observed in all neurons tested with 1-s constant current pulses of varying intensities. An example of instantaneous firing frequency (1/interspike interval) versus injected current plot is shown for the first and last interspike interval (Fig. 4C). Although in control solution the difference between these values was small and skewed toward large injected currents, in apamin solution there was a much more substantial difference throughout the injected current range. The f-I relation slope calculated for the first interspike interval (100 ± 10 Hz/nA) and for steady state firing (58.1 ± 0.7 Hz/nA) evoked by 50–200 pA currents increased, in apamin solution, to 320 ± 20 Hz/nA and 160 ± 20 Hz/nA, respectively (n = 12 cells).

Application of carbachol (50 μM) also increased firing frequency of HMs in response to current injection (Fig. 4D). Spike frequency adaptation was manifested in the presence of carbachol (Fig. 4E), leading to a stronger separation between early and late firing frequencies (Fig. 4F).

In the presence of 100 nM apamin, carbachol (50 μM) had little effect on firing properties (Fig. 5A) as quantified in Fig. 5B where data points for apamin or carbachol tests overlap. The analysis of f-I plots (Fig. 5C) indicates strong similarity between the effects of apamin and carbachol on the AP frequency rise and firing adaptation. Nevertheless, despite occlusion by apamin of carbachol effects on firing, this latter substance was still able to depolarize HMs (8 ± 2 mV; n = 5) without significant change in Rm (89 ± 5%; n = 5). The present data suggest that muscarinic receptor activation could differentially modulate membrane potential and firing properties of HMs.

**Membrane current sensitive to apamin and carbachol**

Apamin-sensitive membrane currents were investigated under voltage clamp conditions. Outward membrane currents were recorded, in the presence of TTX, by delivering depolarizing voltage steps (1-s duration) in the range −40 to +20 mV, whereas Vh was usually −50 mV to minimize contamination by low threshold K+ currents such as I_{fast} (Lape and Nistri 1999). However, tests (n = 8 cells) carried out with −60 or −70 mV Vh yielded similar results to those from the less negative Vh. The standard depolarizing pulse protocol generates a heterogeneous, voltage-dependent outward current (Lape and Nistri 1999), a typical example of which is shown in Fig. 6A. The outward current did not inactivate during the 1-s-long voltage steps and was followed by a tail current. The contribution of the apamin-sensitive current to the total membrane current was examined by adding 100 nM apamin to the extracellular solution. Apamin (which did not change leak conductance; 96 ± 3%) reduced by 36 ± 1% the outward current (Fig. 6B; n = 10) measured 10 ms before the voltage step termination. The apamin-sensitive outward current could then be obtained by subtracting the current recorded in apamin solution...
from the control one. The average $I$-$V$ relation for the apamin sensitive current is plotted in Fig. 6C. Its apparent activation threshold was $-40$ mV from which the current grew monotonically with increasing membrane potential. Note that at $+20$ mV, the value which corresponds to the peak of an action potential, the apamin current had an average cord conductance of 7.62 nS. Of course, this value was calculated when the current was under apparent steady-state conditions following a relatively long depolarization, an experimental condition which cannot approximate the rapid voltage change generated by a single AP.

To characterize the apamin-sensitive current deactivation we studied tail currents following voltage steps to $+20$ mV in the absence or in the presence of apamin (see Fig. 6B). In control conditions tail currents could be fitted by two exponentials with decay time constants of 24 $\pm$ 3 ms and 154 $\pm$ 9 ms at $-50$ mV, respectively ($n = 11$ cells). In the presence of apamin only a fast monoexponential component remained (decay time constant $= 19$ $\pm$ 3 ms; $n = 5$ cells). After current subtraction, the apamin-sensitive tail current was shown to have a monoexponential decay ($140 \pm 20$ ms), which was voltage independent in the range between $-40$ and $-120$ mV (not shown). This observation suggests that deactivation of the apamin-sensitive current was relatively slow and unaffected by membrane potential. Apamin-sensitive tail currents reversed at $-91$ $\pm$ 1 mV ($n = 5$) membrane potential, a value very near $E_K$ ($-95$ mV).

Studying the activation kinetics of the apamin-sensitive current was difficult because of its contamination by the concomitant development of other voltage-dependent currents. To partially circumvent this problem we studied the kinetics of generation of the apamin-sensitive tail currents by applying voltage steps of different lengths. In this case the current flowing at the end of each voltage command should have represented the activation of a certain fraction of apamin-sensitive channels for a given membrane potential. The protocol therefore consisted of delivering fixed-amplitude voltage steps of increasing duration (from 1 to 50 ms) in the absence or the presence of apamin and in measuring the tail currents obtained after current subtraction. The subtracted tail currents were normalized with respect to the one obtained after a 50-ms

FIG. 4. Effect of mAHP block by apamin or carbachol on repetitive firing properties of neonatal HMs. A: responses to the current step (1 s, 200 pA) injected into a HM in standard solution (left) or in 100 nM apamin solution (right). Note large increase in firing frequency. B: plot of instantaneous firing frequency versus time (same HM responses as in A) before (○) and after (●) application of apamin. Note that slight, early spike frequency adaptation in control is replaced by 2 phases of spike frequency adaptation in apamin solution. C: initial (1 isi; triangles) and steady state (ss; circles) $f$-$I$ relations (same neuron as in A and B) before (open circles and triangles) and after apamin (closed circles and triangles) application. D: spike trains elicited by current step injection (1 s, 300 pA) in control or in 50 $\mu$M carbachol solution. Note increase in firing rate. E: plot of instantaneous firing frequency against time (same responses as in D) before (○) and after carbachol (●) application. F: initial (triangles) and steady state (circles) $f$-$I$ relations (same neuron as in D and E) before (open circles and triangles) and after carbachol (closed circles and triangles) application. Note that the depolarizing effect of carbachol was offset by steady current injection to maintain the cell at resting potential.
step and then plotted (Fig. 6E) versus the voltage-step duration (step command to 20 mV). The time course of tail current development was fitted by two exponentials with time constants of 0.7 ± 0.1 and 24 ± 3 ms (n = 5 cells). Note that even 1-ms voltage command was able to generate a measurable fraction of the apamin sensitive current (~20%).

Examples of outward currents recorded before or after adding 50 μM carbachol are shown in Fig. 7, A and B. On average carbachol depressed the outward current by 28 ± 3% (n = 6), a phenomenon associated with the generation of an inward current (40 ± 20 pA). Analysis of tail currents (Fig. 7C) indicated that carbachol blocked the slow component (by 80 ± 10%) strongly and the fast one (by 35 ± 9%) weakly (n = 6). Nevertheless, 50 μM carbachol in the presence of 100 nM apamin could still reduce the steady-state outward current by 28 ± 0.3% (n = 3 cells) as shown in the example in Fig. 7, D and E. In apamin solution the monoexponentially decaying (20 ± 5 ms) tail current was also depressed in amplitude by 20 ± 10% (Fig. 7F).

**FIG. 5.** Carbachol affects repetitive firing properties of HMs via an apamin-sensitive conductance. A: responses to current pulse (1 s, 300 pA) injection in control solution, in presence of 100 nM apamin, and during co-application of 100 nM apamin and 50 μM carbachol. B: plot of instantaneous firing frequency versus time (same responses as in A) for control (○), apamin (□), and coapplication of apamin and carbachol (▲). C: initial (1 isi; open circles, squares, and triangles) and steady state (ss; closed circles, squares, and triangles) I-F relations of the same neuron as in A and B for control (circles), apamin (squares), and coapplication of apamin and carbachol (triangles). Note that the depolarizing effect of carbachol was offset by steady current injection to maintain the cell at resting potential.

**DISCUSSION**

The major novel findings of the present study were that, by using patch clamp recording from rat hypoglossal motoneurons, it was possible to quantify macroscopically the outward current apparently responsible for the mAHP and to demonstrate its modulation by muscarinic receptor activation. In addition, the present patch clamp experiments provided new evidence for the role of the mAHP in spike firing and its regulation by muscarinic receptors.

**mAHP**

Single or repeated action potentials were followed by a series of depolarizing and hyperpolarizing afterpotentials as exemplified in Fig. 1. The fAHP, mAHP, and fADP were present in all HMs. A few cells possessed the sAHP (see also Nishimura et al. 1989; Viana et al. 1993b) or the sADP, which has also been observed in facial motoneurons (Nishimura et al. 1989).

**FIG. 6.** Apamin-sensitive current of HMs. All data are from voltage clamp experiments. A: membrane currents elicited by application of depolarizing voltage steps between −40 and +20 mV (10-mV increments) from −50 mV holding potential in control solution containing TTX. B: set of membrane current traces elicited by the same voltage steps in the same neuron as in A after application of 100 nM apamin. Note reduction in steady-state outward current. C: average I-V relation (n = 5 cells) of apamin-sensitive membrane currents obtained after subtracting current traces recorded in apamin solution from those recorded in control solution. Note that the current activation threshold is near −30 mV. D: superimposed tail currents recorded at the end of +20 mV voltage steps as the membrane potential was returned to −50 mV (same neuron as in A and B in control or in the presence of 100 nM apamin). Note shortening of tail current in apamin solution. E: plot of apamin-sensitive tail current (obtained by subtracting tail currents recorded in apamin solution from those recorded in control solution) versus voltage step to +20 mV duration. The time course of apamin-sensitive tail current development is well fitted by 2 exponentials (0.7 ± 0.1 and 24 ± 3 ms; n = 5 cells). Currents were normalized with respect to the one obtained by a 50-ms depolarizing step.
The HM mAHP was qualitatively similar to the one recorded with sharp electrodes from brain stem (Chandler et al. 1994; Mosfeldt Laursen and Rekling 1989; Nishimura et al. 1989; Sah and McLachlan 1992; Viana et al. 1993b) or spinal (Walton and Fulton 1986; Zhang and Krnjevic 1987) motoneurons. This similarity demonstrates that whole-cell patch clamping allowed us to measure this response without introducing artifacts inherent to the recording technique. The mAHP was completely blocked by Ca$^{2+}$-free solution or apamin, and it reversed at membrane potential near the predicted $E_{K^{-}}$. All these observations indicate that mAHP was a Ca$^{2+}$-dependent K$^{+}$ conductance. Its sensitivity to apamin, a very selective blocker of SK channels (for review see Sah 1996), suggests that the Ca$^{2+}$-dependent K$^{+}$ conductance responsible for this phenomenon was probably mediated by SK channels and turned on by Ca$^{2+}$ entry after one (or more) AP. In fact, the lack of effect of apamin on resting potential or leak conductance shows that such a Ca$^{2+}$-dependent K$^{+}$ conductance was inactive at resting levels of intracellular Ca$^{2+}$.

Apamin sensitive current

The outward current selectively inhibited by apamin may be termed $I_{AHP}$ (see Sah 1996). On HMs this represented about one third of the total outward current induced by membrane depolarization and deactivated slowly. For 1-s-long membrane depolarization to +20 mV the cord conductance of $I_{AHP}$ (under apparently steady-state conditions) was 7 ± 2 nS. The corresponding conductance value for the slow K$^{+}$ current ($I_{slow}$; values taken from Fig. 2A in Lape and Nistri 1999) was 14 nS, whereas the fast transient current ($I_{fast}$) was strongly inactivated at this time point. Thus during sustained depolarization $I_{slow}$ generated a membrane shunt considerably larger than $I_{AHP}$. Note that in addition to these K$^{+}$ currents there was a residual, unidentified outward current sensitive to muscarinic agents as discussed below. Because the largest component of the total outward steady current was apparently made up of $I_{slow}$ (which deactivated with a faster time course; Lape and Nistri 1999), it was difficult to study $I_{AHP}$ in isolation. For this reason kinetic parameters pertaining to $I_{AHP}$ were obtained by analyzing the current (and especially its slow tail) obtained after subtraction. The $I$-$V$ relation of $I_{AHP}$ indicated an apparent activation threshold at about −40 mV; its nonlinear voltage dependence in the −40/−10 mV range might have reflected the strong voltage dependence of Ca$^{2+}$ conductance activation (Hille 1992). The time course of $I_{AHP}$ development was biexponential. This latter property probably reflects the multifactorial process underlying $I_{AHP}$ generation and might have been due to phased recruitment of SK channels by increasingly larger amounts of intracellular Ca$^{2+}$ diffusing over a wide cytoplasmic area.

It is noteworthy that membrane depolarization as short as 1 ms could elicit a measurable $I_{AHP}$. It is suggested that even very short voltage changes (as brief as a single AP, normally lasting 1–3 ms) can trigger Ca$^{2+}$ entry sufficient to turn on an adequate number of SK channels to generate an mAHP. Similar observations have been obtained with ganglion neurons (Lancaster and Pennefather 1987).

It is useful to assess the relative contribution of $I_{fast}$, $I_{slow}$, and $I_{AHP}$ to a single action potential: by assuming a spike lasting 3 ms the calculated cord conductances are 4 nS (from Fig. 3C of Lape and Nistri 1999), 1 nS (from Fig. 2A of Lape and Nistri 1999), and 3 nS (present study; see Fig. 6E), respectively. Even if calculations based on responses to a fast step pulse cannot fully take into account the dynamic changes in spike shape, it appears that the main K$^{+}$ conductance activated during a single spike was $I_{fast}$. The role of $I_{AHP}$ would probably be more conspicuous after the spike itself because $I_{AHP}$ activation continued because of its dependence on delayed Ca$^{2+}$ entrance. This property makes $I_{AHP}$ the most suitable mechanism to generate the mAHP as $I_{fast}$ and $I_{slow}$ deactivate with a more rapid time course (Lape and Nistri 1999).

It is interesting that $I_{AHP}$ deactivation (observed as monoeponential decay time constant of the apamin-sensitive tail current) showed no voltage dependence, thus suggesting that the membrane conductance underlying $I_{AHP}$ was probably voltage independent. $I_{AHP}$ with similar kinetic properties was described in sympathetic neurons (Cassell and McLachlan 1987; Goh and Pennefather 1987), vagal motoneurons (Sah and McLachlan 1992), trigeminal motoneurons (Chandler et al. 1994), and cortical neurons (Schwindt...
et al. 1992). The present data will help reconstructing HM firing behavior with computer-generated modeling based on experimentally acquired data.

Effects of muscarinic receptor activity on mAHP

The mAHP of HMs is a target for neuromodulation by 5-HT or norepinephrine (Bayliss et al. 1995; Parkis et al. 1995). Both substances do not act directly on the mAHP underlying conductance but operate indirectly by either inhibiting Ca\(^{2+}\) currents in the case of 5-HT (Bayliss et al. 1995) or reducing leak conductance (and activating an inward current) in the case of norepinephrine (Parkis et al. 1995). The present study shows that the mAHP (and \(I_{\text{AHP}}\)) is also a target for muscarinic receptor activity. Carbachol or muscarine reduced mAHP amplitude without changing its rise and decay times, suggesting that muscarinic receptors apparently led to inhibition of a fraction of \(I_{\text{AHP}}\) channels. It should be pointed out that previous studies on cortical neurons have reported that muscarinic receptors usually block the sAHP (Schwindt et al. 1988, 1992) while sparing the mAHP. On the other hand, as in the case of HMs, the mAHP of hippocampal neurons is reduced by carbachol (Fisman et al. 1991; Storm 1989; Williamson and Alger 1990; Zhang and McBain 1995). It is however clear that in hippocampal cells several conductances participate in the generation of mAHP (Storm 1989) and that one of them, the so-called \(I_{M}\), may be the main target for the blocking action by carbachol on the mAHP (Halliwell and Adams 1982). It seems unlikely that on HMs \(I_{M}\) was responsible for generating the mAHP because \(I_{M}\) is not Ca\(^{2+}\) dependent and has slow activation kinetics (Adams et al. 1982, Brown and Selyanko 1985) which preclude its turning on by a single AP. The present study thus indicates the mAHP as a novel site of action for muscarinic receptors of hypoglossal motoneurons. Furthermore, atropine enhanced the mAHP amplitude, suggesting that under the recording conditions of the slice preparation there was background release of acetylcholine sufficient for partial inhibition of the mAHP.

Our data however indicate that, in addition to \(I_{\text{AHP}}\), other K\(^{+}\) currents of HMs were modulated by muscarinic receptors. In fact, in the presence of apamin when \(I_{\text{AHP}}\) should have been completely blocked, carbachol could still reduce a component of the sustained outward current. The multiple sites of carbachol action on HMs were confirmed in experiments under current clamp conditions, as a reduction in mAHP and membrane depolarization could be differentially antagonized by apamin. Although block of a variety of K\(^{+}\) conductances contributes to the carbachol-evoked depolarization (Benardo and Prince 1982; Storm 1990; Womble and Moises 1992), it is possible that enhancement of a Ca\(^{2+}\)-dependent nonspecific cationic current is also a factor leading to membrane depolarization (Colino and Halliwell 1993). Unlike the case of CA1 hippocampal cells (Figenschou et al. 1996), the present study did not observe any change in action potential duration or threshold in the presence of carbachol.

Repetitive firing

Adult HMs in brain stem slices show three distinct phases of spike frequency adaptation (Sawczuk et al. 1995, 1997). In most cases HMs of neonatal rats display fast spike adaptation or, in a minority of cases, firing acceleration (Viana et al. 1993b, 1995). The present study found no evidence for spike frequency acceleration, whereas fast adaptation was the most common response (a minority of cells had a regular firing pattern). The differences might be due to postnatal developmental changes (as the present results were obtained from younger rat cells that often show fast adaptation; Viana et al. 1995) or to the recording conditions (sharp versus patch electrodes; blind recording versus visually identified motoneurons; large current pulses versus weaker ones). In the present investigation the crucial role of the mAHP in firing behavior became immediately apparent after the mAHP was blocked by apamin, carbachol, or Ca\(^{2+}\)-free solution. Strong firing adaptation was readily manifested as a result. Previous studies have shown the importance of the mAHP in controlling repetitive firing in different neurons (Baldissera and Gustafsson 1974; Chandler et al. 1994; Kernel and Sjoholm 1973; Nishimura et al. 1989; Powers et al. 1999; Storm 1990; Viana et al. 1993b). Whenever the mAHP was present to hyperpolarize the membrane potential of HMs, the duration of membrane potential sojourns at depolarized level became inadequate for full activation or inactivation of various voltage-dependent conductances (for instance compare AP duration lasting \(\sim 3\) ms with activation time constants of \(8 \pm 3\) ms for \(I_{\text{fast}}\) and \(18 \pm 3\) ms for \(I_{\text{slow}}\); Lape and Nistri 1999). These kinetic properties thus prevented the onset of the adaptation process. The present results therefore demonstrate that the mAHP of neonatal HMs had the fundamental property of maintaining a slow firing frequency (with a relatively regular pattern) but, at the same time, it could not be the principal component for spike adapting properties.

The fact that strong spike frequency adaptation appeared when the mAHP was fully suppressed by apamin raises the question of the relative contribution by different conductances to the control of repetitive firing. In the presence of apamin (either alone or plus carbachol which should have also blocked \(I_{M}\) or leak conductance as discussed earlier), fast adaptation probably developed because of the kinetic properties of \(I_{\text{fast}}\). Slow adaptation (which developed over a matter of hundreds of milliseconds) presumably relied on \(I_{\text{slow}}\) especially as the baseline membrane potential, elevated during the spike train, should have facilitated persistent \(I_{\text{slow}}\) activation. Modulation of mAHP by neuromodulators like muscarine, 5-HT (Bayliss et al. 1995), or norepinephrine (Parkis et al. 1995) suggests that HM firing could change during different behavioral states associated with various degrees of activity of cholinergic, serotonergic, or noradrenergic pathways. These transmitters might thus act via dynamic alterations in the role of various K\(^{+}\) conductances to the total outward current.

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