Endogenous Firing Patterns of Murine Spiral Ganglion Neurons

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Mo, Zun-Li and Robin L. Davis. Endogenous firing patterns of murine spiral ganglion neurons. J. Neurophysiol. 77: 1294–1305, 1997. Current-clamp recordings with the use of the whole cell configuration of the patch-clamp technique were made from postnatal mouse spiral ganglion neurons in vitro. Cultures contained neurons that displayed monopolar, bipolar, and pseudomonopolar morphologies. Additionally, a class of neurons having exceptionally large somata was observed. Frequency histograms of the maximum number of action potentials fired from 240-ms step depolarizations showed that neurons could be classified as either slowly adapting or rapidly adapting. Most neurons (85%) were in the rapidly adapting category (56 of 68 recordings). Measurements of elementary properties were used to define the endogenous firing characteristics of both neuron classes. Action potential number varied with step and holding potential, spike amplitude decayed during prolonged depolarizations, and spike frequency adaptation was observed in both rapidly and slowly adapting neurons. The apparent input resistance, spike amplitude decrement, and instantaneous firing frequency differed significantly between rapidly and slowly adapting neurons. Inward rectification was evaluated in response to hyperpolarizing constant current injections. Present in both electrophysiological classes, its magnitude was graded from neuron to neuron, reflecting differences in number, type, and/or voltage dependence of the underlying channels. These data suggest that spiral ganglion neurons possess intrinsic firing properties that regulate action potential number and timing, features that may be crucial to signal coding in the auditory periphery.

INTRODUCTION

The sensory cells of the cochlea are innervated by two major classes of primary auditory neurons that compose the spiral ganglion. Type I neurons receive synaptic input from inner hair cells that mediate the initial stages of auditory perception, whereas type II neurons, whose function is currently unknown, innervate outer hair cells (Dallos 1992; Hudspeth 1989; Kelly 1984; Kiang 1984; Kiang et al. 1982; Kim 1984; Perkins and Morest 1975; Ryugo 1992; Spoendlin 1973). Thus, analogous to other sensory modalities such as the somatosensory or visual systems, neurons that innervate different receptor types reside within the same ganglion.

Beyond the two categories of spiral ganglion neurons, which are based on peripheral innervation patterns, further subclassifications have been observed. Electrophysiological studies have shown that type I neurons do not all display identical firing properties in vivo. In addition to differences in characteristic frequency, they show graded thresholds (Liberman 1978), differently shaped rate-intensity functions (Kiang et al. 1965; Winter et al. 1990), and a wide range of spontaneous activity (Kiang et al. 1965; Liberman 1978; Schmidt 1989) that can be classified into three discrete groups (Liberman 1978). These characteristics could result from multiple sources, such as mechanical nonlinearities, synaptic regulation of neuronal firing, and endogenous electrical properties of the neurons themselves (Davis 1996; Evans 1992; Javel 1986; Kiang 1990; Kiang et al. 1986; Ruggero 1992). Immunohistochemical localization of molecules such as neurofilaments (Romand et al. 1990), osteopontin (Lopez et al. 1995), neuropeptide Y, and neuron-specific enolase (Anniko et al. 1995) has further shown the inherent complexity of the spiral ganglion. These molecules are differentially localized to subclasses of primary auditory neurons that do not correspond to the type I/type II categorization. Some of these differences may be associated with activity-regulated expression, such as that observed with Fos and Jun proteins (Morgan and Curran 1991); yet others could be due to subtle subdivisions within the peripheral innervation-based classification (Romand et al. 1990).

Although the molecular studies and in vivo electrophysiology suggest that spiral ganglion neurons have nonuniform characteristics that contribute differentially to the coding of auditory information, little is known about their endogenous firing properties. This is a critical parameter to determine, because electrical signals from the periphery are shaped by postsynaptic membrane currents as well as by synaptic connections (Llinás 1988). On the basis of what is known about primary neurons in other sensory systems (Perl 1992; Shapley and Perry 1986), one would expect to observe distinct electrophysiological features that distinguish neurons within the auditory periphery that innervate different receptor types.

To begin to address the issue of whether spiral ganglion neurons possess heterogeneous intrinsic firing patterns, we made whole cell current-clamp recordings from primary auditory neurons separated from their peripheral targets in vitro. These studies showed unequivocally that postnatal murine primary auditory neurons do not display uniform responses to injected currents. Instead, we identified two general neuron classes on the basis of action potential adaptation. The largest proportion of cells fired only a few spikes to a prolonged depolarization, thus adapting rapidly to the stimulus. The remaining neurons fired multiple spikes, showing slow adaptation. Other parameters, such as spike amplitude decrement, spike frequency adaptation, and inward rectification were examined in both neuronal classes and were found to be graded in magnitude. These results suggest that subcategories of postnatal spiral ganglion neurons differentially influence the time course and magnitude of the receptor potential signals they transmit to the brain.

METHODS

Tissue culture

Spiral ganglia, removed from the surrounding cochlear tissues, were taken from neonatal CBA/J mice ranging in age from 1 to 6 postnatal days of development and were plated as explants in...
culture dishes coated with poly-L-lysine (Sigma, P-9155). Neuronal cultures were maintained in growth medium consisting of Dulbecco’s modified Eagle’s medium (Sigma, D-6171) supplemented with 10% fetal bovine serum (Sigma, F-2442), 4 mM l-glutamine (Sigma, G-6392), and 0.1% penicillin-streptomycin (Sigma, P-0781). The growth medium in some of the neuronal cultures (from which 17 recordings were included in the total data) was supplemented with neurotrophins (5–10 ng/ml of brain-derived neurotrophic factor alone or in combination with nerve growth factor). Although more subtle effects may be present, no systematic differences were noted in the basic firing properties of neurons maintained in either supplemented or unsupplemented media. Therefore data from these different conditions were pooled.

Cultures consisted of neurons, identified with neurofilament antibodies (see below), as well as satellite cells (Fig. 2A). Therefore we limited our electrophysiological observations to cells that showed a large and rapidly inactivating inward current in whole cell voltage clamp. Neurons could also be distinguished from non-neuronal cells when viewed with Hoffman optics because of their large round somata, which contained a prominent nucleus and a single nucleolus.

**Immunofluorescence**

To examine the morphology of the neurons from which recordings were typically made, >40 cultures of mouse tissue maintained in different combinations of neurotrophins were stained with neurofilament 200 monoclonal antibody (NF200; Sigma, N-0142). There was no difference between the basic morphological characteristics (relative soma size and shape) of neurons maintained in unsupplemented growth medium and those exposed to added neurotrophins. This was examined from neurons exposed to nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and combinations of these neurotrophins.

Tissues were fixed in 100% methanol at −20°C for 6 min; cultures were then incubated for 1 h in a 5% solution of serum of the species in which the secondary antibody was raised, or in instant nonfat dry milk. The primary antibody was applied and left for 1 h at room temperature. A fluorescent-conjugated secondary antibody was subsequently applied for 1 h. Between each step, the preceding solution was removed by washing 3 times in 0.1 M phosphate-buffered saline (pH 7.4). For control cultures, tissue was treated identically except that the primary antibody was not included. A Zeiss Axiosvert 135 microscope with epifluorescence illumination and Zeiss ×10 and ×40 (long working distance) objectives was used to visualize either fluorescein isothiocyanate isomer I-conjugated or tetramethyl-rhodamine isothiocyanate-conjugated secondary antibodies. The fluorescein isothiocyanate isomer I filter set was composed of (in nm) 505LP dichroic mirror, 480–500 excitation filter, and 535–540 barrier filter. The tetramethyl-rhodamine isothiocyanate filter set was composed of (in nm) 565LP dichroic mirror, 540–550 excitation filter, and 605–550 barrier filter.

**Electrophysiology**

The whole cell configuration of the patch-clamp technique was used to obtain current-clamp recordings from primary auditory neurons in vitro (Hamill et al. 1981). Extensive voltage-clamp recordings were not made from these cells because the long neurites precluded adequate voltage control. Furthermore, whole cell rather than perforated patch-clamp recordings were utilized to attain optimal electrical access to the cell soma. Electrodes were pulled on a two-stage vertical puller (Narishige, PP-83) and the shafts were coated with Sylgard (Dow Corning) to reduce the pipette capacitance. Just before use, electrode tips were fire-polished (Narishige MF-83 microforge); electrode resistances typically ranged from 2 to 5 MΩ in standard pipette and bathing solutions (see below). Pipette offset current was zeroed immediately before the cell membrane was contacted; current-clamp measurements were made exclusively with low-resistance electrodes to avoid the need for bridge balance compensation.

Recordings were considered acceptable when they met the following criteria: stable holding potentials, low noise levels, discernible membrane time constant on step current injection, and overshooting action potentials (magnitude of ±70 mV for the 1st action potential in a burst). If any of these parameters changed during an experiment, the remaining data were not analyzed. Furthermore, endogenous membrane properties were routinely retested during the course of a recording. This precaution was especially relevant to investigations of inward rectification, which showed differences in magnitude between recordings and at different holding potentials in the same recording.

For all characterizations of firing properties, an action potential was defined as a rapid and transient depolarization with a clear inflection preceding solution was removed by washing 3 times in 0.1 M phosphate-buffered saline (pH 7.4). For control cultures, tissue was treated identically except that the primary antibody was not included. A Zeiss Axiosvert 135 microscope with epifluorescence illumination and Zeiss ×10 and ×40 (long working distance) functions did not differ substantially when aligned at their maximum and minimum values (0 current was aligned with the −60 mV holding potential for the minimum level).
not form synapses with one another in vitro. Therefore the electrical properties examined herein most likely reflect the endogenous activity of spiral ganglion neurons devoid of synaptic connections.

To compare the voltage-dependent properties of one neuron versus another, measurements were expressed as a function of voltage level attained, rather than injected current. The use of this physiologically relevant parameter does not distort any of the neuronal characteristics described in this paper; for example a comparative analysis of interspike interval is shown in Fig. 1B. As expected, the match between the two functions is not exact, yet their shape is independent of whether current or voltage is used for the abscissa. Thus step potential refers to the new membrane potential achieved in response to a 240-ms depolarizing constant current injection, and holding potential refers to the voltage maintained with long-term (generally hyperpolarizing) constant current injections.

Depolarizations were measured at the steady-state level close to the termination of the depolarizing current injection. However, when this procedure is used, some uncertainty exists in the relatively few cases in which action potentials were elicited throughout the current injection (5 neurons, each over a maximum range of ~20 mV). Because these neurons displayed fewer spike numbers at higher depolarizations, measurements could be made at flanking voltage levels (i.e., Fig. 3B, top and bottom traces). In such instances the intermediate voltages were estimated with the use of two different procedures: 1) interpolating between two known values at which measurements could be made, and 2) obtaining an average voltage value over the 240-ms depolarization (Fig. 3B, dashed line). Interpolated and averaged values corresponded closely to one another, each showing smooth transitions from measured to estimated values in current-voltage relationships.

A standard set of solutions was used to approximate physiological conditions. The basic internal solution was composed of (in mM) 112 KCl, 2 MgCl₂, 0.1 CaCl₂, 11 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, and 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), pH 7.4, and was either used alone or with 2 mM ATP. ATP was added to stock solution on the day of the recording and then the pH was readjusted to 7.4. We saw no obvious differences in recordings made with either pipette solution, suggesting, but not ruling out, that neuronal firing properties were not affected by ATP-regulated currents. Neurons were exposed to the following bath solution (composition, in mM): 137 NaCl, 5 KCl, 1.7 CaCl₂, 1 MgCl₂, 17 glucose, 50 sucrose, and 10 HEPES, pH 7.5 (Davis 1996).

Recordings were made from neurons maintained for 1–13 days in vitro at room temperature (19–22°C) with the use of an Axopatch 200 (Axon Instruments) patch-clamp amplifier. Data were digitized with an Indec IDA 15125 interface in an IBM-compatible personal computer; the programs for data acquisition and analysis were written in Borland C++ and Microsoft Visual Basic (generously contributed by Dr. Mark R. Plummer). Each segment of data (400 ms in duration) was digitized at 5 kHz and filtered at 1 kHz.

RESULTS

Recordings were made from 98 murine neurons, of which 70 were sufficiently complete to be examined in detail. Evaluations of neuronal gross morphology by immunostaining cultures identical to those used for electrophysiology showed cells with extensive processes. As shown in other studies (Mou and Davis 1996), NF200 antibodies colocalized with neuron specific enolase and high-affinity tyrosine kinase receptor antibodies.

The majority of cultured neurons were monopolar, having only a single neurite projecting from the cell body, presumably because of regeneration of one rather than two processes. However, a significant proportion of the neurons that

FIG. 2. Spiral ganglion neurons maintained in culture with surrounding satellite cells showed characteristic morphological features. A: neurons immunostained with neurofilament 200 monoclonal antibody (NF200) were viewed with Hoffman optics and epifluorescence. The bright fluorescence highlighted neurons that were surrounded by satellite cells. Arrow: cluster of neurons. Arrowhead: unstained nucleus containing a prominent nucleolus. B: bipolar neuron with processes that emanated from either side of the soma immunostained with NF200. C: pseudomonopolar neuron with processes originating from the same side of the cell somata immunostained with NF200. Process at right was not truncated, but projected out of the focal plane. D: example of a large neuron (large arrow) next to a standard-sized neuron (small arrow). Neurons from postnatal day 6 mice were maintained for 7 days in vitro with either 2 ng/ml neurotrophin-3 alone (C), or 2 ng/ml brain-derived neurotrophic factor in combination with 2 ng/ml neurotrophin-3 (A, B, and D). Calibration bar (D, bottom right) represents 40 μm for all panels.
had two processes (Fig. 2B) displayed the classic bipolar morphology of spiral ganglion neurons with neurites emanating from either end of an ovoid-shaped soma (Berglund and Ryugo 1987; Brown et al. 1988). Neurons were also observed that displayed a pseudomonopolar-like morphology (Fig. 2C) similar to that of type II neurons in cat (Kiang et al. 1984), and these were found in both classes of murine spiral ganglia (Berglund and Ryugo 1987). Although neuronal diameter was not examined quantitatively, in most cases the somata were of uniform size. We did, however, observe a low percentage of cells that had notably large somata (Fig. 2D), possibly corresponding to the large type II neurons described by Berglund and Ryugo (1987). Except for the striking size of the large neurons, cell body morphology was not a reliable indicator of neuronal class because bipolar and pseudomonopolar morphology does not discriminate between murine type I and type II spiral ganglion cells. Nevertheless, the presence of bipolar and pseudomonopolar somata bodies, as well as the distinctly large neuronal somata observed in vitro, indicated that neurons in culture displayed morphological profiles similar to those of cells observed in vivo (Berglund and Ryugo 1987).

Recordings were made from random spiral ganglion neurons in vitro, regardless of size or morphology. From current-clamp evaluations of murine spiral ganglion neurons, we determined that firing characteristics were not uniform. Two broad categories were distinguished: rapidly adapting neurons that fired one or only a few action potentials in response to a depolarizing constant current injection, and slowly adapting neurons that fired multiple action potentials at similar step potentials (Fig. 3). The greatest difference between these two neuron classes was observed with current injections that changed the membrane potential to voltages ranging from −40 to 0 mV. For example, a rapidly adapting neuron (Fig. 3A, 2nd trace) fired only 3 action potentials when the membrane potential was stepped to −20 mV, whereas a slowly adapting neuron (Fig. 3B, 2nd trace) fired 20 action potentials at a similar potential. This difference was not as apparent at lower or higher voltage levels; for example, the slowly adapting neuron fired only two more action potentials than the rapidly adapting neuron at relatively hyperpolarized potentials (Fig. 3, A and B, bottom traces).

Spiral ganglion neurons, regardless of whether they displayed rapidly or slowly adapting firing properties, all demonstrated an initial increase in the action potentials generated with small, graded depolarizations. Action potential numbers reached a maximum at moderately depolarizing current injections, then either declined or remained constant with further increases in the magnitude of the depolarization (Fig. 4, insets). Action potentials fired at each depolarizing constant current injection were also influenced by the holding potential. The action potential number obtained from the same slowly adapting neuron (black triangles) held at −60 mV (Fig. 4A, inset) and −80 mV (Fig. 4B, inset) decreased from 20 to 16. Similarly, the number in the same rapidly adapting neuron (gray diamonds) held at −60 mV (Fig. 4A, inset) and −80 mV (Fig. 4B, inset) also decreased (from 4 to 2).

The maximum number of action potentials fired in response to 240-ms depolarizing current injections (APmax) was evaluated from multiple recordings to examine the number and distribution of neuronal firing patterns. As shown in Fig. 4A, when graded potentials were tested from −60 mV, two clear response categories were evident. These nonoverlapping distributions were used to distinguish rapidly adapting neurons from slowly adapting neurons for the purpose of subsequent analysis. These separate classes were also observed at more hyperpolarized holding potentials. The ranges were partially overlapping at −80 mV (Fig. 4B), however, because the numbers of action potentials declined as the holding potential was decreased. The apparent input resistance (Rin), measured close to zero injected current, was significantly different for each category (P < 0.01). Slowly adapting neurons had an average Rin of 209 ± 23.4 (SE) MΩ (N = 10), whereas rapidly adapting neurons had an average Rin of 297 ± 27.5 MΩ (N = 21).

To define these two electrophysiological categories further...
ther, endogenous firing characteristics were analyzed and compared for each group. Time- and voltage-dependent features (amplitude decrement, spike frequency adaptation, instantaneous firing frequency, and inward rectification) showed a continuum in both neuronal classifications. Two of these parameters, spike amplitude decrement and instantaneous firing frequency, differed significantly between rapidly and slowly adapting neurons; the others displayed greater variation within each category than between them.

Successive action potentials declined in amplitude until only small oscillations were observed in both rapidly and slowly adapting neurons (Fig. 3, A and B). This amplitude decrement became more pronounced as the membrane potential was stepped to depolarized levels with constant current injections (Fig. 5, A and B). Spike amplitudes were measured from the peak of the voltage excursion to the nadir of the subsequent afterhyperpolarization. In most cases, the greatest difference in amplitude was observed between the first and second spikes for slowly adapting neurons. In the case of rapidly adapting neurons, however, these were the only measurements made because very few spikes were fired by these cells.

There was a linear relationship between test potential and the degree of decrement in the first two action potentials (Fig. 5, C and D). In the two cases illustrated, action potential amplitude declined more abruptly for the rapidly adapting neuron than for the slowly adapting one. Evaluations made from multiple cells (Fig. 5, E and F) indicated that rapidly adapting neurons displayed significantly greater amplitude decrement at similar step potentials. The decrease in amplitude at a step potential of $-10 \text{ mV}$ was $-44.5 \pm 3.7 \text{ mV}$ and $-33.0 \pm 4.1 \text{ mV}$ for rapidly and slowly adapting neurons, respectively ($P < 0.05$).

Rapidly and slowly adapting neurons also displayed spike frequency adaptation, characterized by a decline in instantaneous frequency over the course of step current injection.
Figure 5. Spike amplitude declines over the time course of a depolarizing current injection for rapidly adapting (A, C, and E) and slowly adapting (B, D, and F) neurons. 

**A**: rapidly adapting neuron maintained at −60 mV with hyperpolarizing current of 20 pA, from which depolarizing current injections of 130, 170, 210, 250, 360, 490, and 650 pA produced step potentials of −28, −22, −20, −18, −13, −8, and −4 mV, respectively. Amplitude decrement increases as the membrane is stepped to more depolarized levels.

**B**: slowly adapting neuron maintained at −60 mV with depolarizing current of 10 pA, from which depolarizing current injections of 40, 110, 230, 350, 470, 630, and 950 pA produced step potentials of −47, −40, −30, −19, −4, +4, and +25 mV, respectively. Amplitude declined more precipitously at depolarized levels.

**C** and **D**: amplitude difference between the 1st and 2nd action potentials plotted as a function of step potential from −60 mV. Constant current injections ranged from 40 to 700 pA (**C**) and from 20 to 500 pA (**D**). The relationship between the spike amplitude and membrane potential was well fitted with linear functions.

**E** and **F**: superimposed linear fits from rapidly adapting (N Å 12) and slowly adapting (N Å 9) neurons. Note that the amplitude differences were greater for rapidly adapting neurons at comparable step potentials. Lines in **C**–**F** were fitted with least-square fits.

Figure 3B, bottom trace, illustrates this point clearly, because the interval between the third and fourth spikes was much greater than the interval between the first and second spike. This property was most prevalent at hyperpolarized test potentials; careful examination at more depolarized levels, however, showed that the first and second spikes occurred in more rapid succession than subsequent ones. Typical examples of instantaneous frequency plotted as a function of action potential number are shown in Fig. 6, A and B, for a rapidly and a slowly adapting neuron, respectively.

Instantaneous frequency declined at each test potential evaluated for rapidly adapting neurons (Fig. 6A). Instantaneous frequency decrement was limited to the initial spike intervals of slowly adapting neurons, then remained relatively constant for subsequent action potential intervals (Fig. 6B). Because rapidly adapting neurons fired far fewer action potentials than slowly adapting ones, systematic comparison of the two types was accomplished by analyzing the interval between the first two spikes as a function of increasing depolarizing current injections (Fig. 6, C and D). Interspike interval declined abruptly at hyperpolarizing test potentials but remained relatively stable at more depolarized levels. Data from multiple recordings were fitted with the sums of two exponentials and superimposed to show the range of measured values for rapidly adapting (N Å 18; Fig. 6E) and slowly adapting neurons (N Å 7; Fig. 6F). Slowly adapting neurons displayed a significantly steeper voltage dependence than rapidly adapting ones; the interspike interval at −10 mV was 12.3 ± 0.6 ms and 9.4 ± 0.4 ms for rapidly and slowly adapting neurons, respectively (P < 0.01).

Evaluation of responses to hyperpolarizing current injections revealed that both rapidly and slowly adapting neurons displayed graded amounts of inward rectification at voltages more negative than the holding potential (Figs. 7 and 8, respectively). For example, although some rapidly adapting neurons displayed almost ohmic behavior (Fig. 7A), others showed relatively large nonlinearities over similar voltage ranges (Fig. 7B). Unlike the rapidly adapting neurons, all slowly adapting neurons showed some inward rectification when stepped to negative potentials from a holding potential of −80 mV; the magnitude, however, was also graded from neuron to neuron (Fig. 8, A and B).

Inward rectification induced from holding potentials of −60 and −80 mV was compared between neurons at a single
level of current injection (300 pA) and quantified by sub-
tracting the steady state from the peak voltage change (as
indicated in Figs. 7 and 8, and respectively). Both
rapidly and slowly adapting neurons (Fig. 9, A and B, gray
diamonds and filled triangles, respectively) showed a range of
inward rectification. There was no significant difference
between the two electrophysiological classes at either of
the holding potentials. The voltage difference at 300-pA
hyperpolarizing current injections from a step potential of
−60 mV was 40.5 ± 7.1 mV and 34.4 ± 4.0 mV for rapidly
and slowly adapting neurons held at −60 mV (P > 0.4).
The voltage difference at 300-pA hyperpolarizing current
injection from a membrane potential of −80 mV was
33.1 ± 5.5 mV and 31.6 ± 4.2 mV (P > 0.8).

The heterogeneity of inward rectification at similar hold-
ing potentials was examined with current-clamp experiments
to quantify the voltage dependence and magnitude of the
hyperpolarizing responses from a series of holding poten-
tials. Data from two different neurons are shown in Fig. 10,
A and B, in which the difference between peak and steady-
state voltage is plotted as a function of hyperpolarizing cur-
rent injection from a series of holding potentials. In both
cells, the difference between the peak and steady-state mea-
surements was maximal for test hyperpolarizations delivered
from a holding potential of −40 mV (Fig. 10A, A and B, ).
A clear distinction could be made between the two cells,
however, based on the amount of inward rectification seen
in response to voltage changes elicited from more negative
holding potentials. For the neuron illustrated in Fig. 10A,
inward rectification declined precipitously when the holding
potential was increased to −60 mV ( ). Responses of the
neuron shown in Fig. 10B showed a much different voltage
dependence with the amount of inward rectification still
observable even from a holding potential of −100 mV. Measure-
ments from multiple neurons were compared by fitting lines to
end points from each series of test pulses (as indicated in Fig. 10, A and B). Two general response patterns were seen: those in which inward rectification in-
creased between holding potentials of −60 and −40 mV
(N = 5; Fig. 10C) and those in which inward rectification
was maximal at holding potentials of −60 mV or less (N =
FIG. 7. Current-voltage relationships for 2 rapidly adapting neurons show different magnitudes of inward rectification. A: neuron with AP max Å 1 was held at −80 mV with a hyperpolarizing constant current injection of 260 pA. Note that there is little difference between the initial (▲) and steady-state (●) voltage for any amount of injected current. B: neuron with AP max Å 1 was held at −80 mV with a hyperpolarizing constant current injection of 100 pA. Note the substantial inward rectification when comparing the initial (▲) and steady-state (●) voltages.

6; Fig. 10D). Furthermore, in the six cases in which inward rectification plateaued at −60 mV, the slopes clearly varied in magnitude. Voltage-clamp recordings from acutely dissociated spiral ganglion neurons (which have no processes) have confirmed the presence of inward rectifying currents with graded magnitudes (unpublished data).

DISCUSSION

The present study was designed to investigate the elementary firing properties of spiral ganglion neurons isolated from their central and peripheral targets. Evaluations of neurons from neonatal mice showed graded and complex signaling characteristics when neurons were examined with whole cell current-clamp. The number of spikes fired in response to depolarizing step potentials revealed two broad classes of neuron: those with rapid adaptation and those with slow adaptation to prolonged depolarizations. These two classes were further distinguished by significant differences in $R_m$, spike amplitude decrement, and interspike interval. Values of two other parameters, spike frequency adaptation and inward rectification, varied within each electrophysiological class.

It is not surprising that two different neuronal firing categories were observed in primary auditory neurons, because it has long been known that the spiral ganglion consists of two major classes of cells (Perkins and Morest 1975; Spoendlin 1973). Correlations between the electrophysiological classes observed in the present study with the two spiral ganglion categories are premature, however, because somata morphologies of type I and type II murine neurons were relatively indistinguishable in vivo (Berglund and Ryugo 1987) and thus could not be used as an indication of neuron classification in vitro. Furthermore, the culture conditions may select for one class or subclass of neuron such that normal distributions observed in vivo are altered by selective survival in vitro. Thus the high percentage of rapidly adapting recordings (85%) does not necessarily reflect type I neurons despite their high composition in adult (90–95%) (Spoendlin 1973) and neonatal (~75%) (Chiong et al. 1993) animals. Nevertheless, we have indications that
some of the cells that displayed slowly adapting features had large-sized neuronal somata (unpublished observations), possibly corresponding to the distinctively large murine spiral ganglion neurons with type II-like innervation patterns described by Berglund and Ryugo (1987). This class of cells within the spiral ganglion most likely represents type III neurons, which were characterized in an ultrastructural study (Romand and Romand 1987). Another possibility, although it is less likely because the size differences did not appear graded, is that the large-sized neurons observed in vitro may instead correspond to a subclass of type I neurons. Soma size has been shown to vary systematically with coding frequency (Liberman and Oliver 1984) and cochlear innervation (Nadol et al. 1990).

The presence of rapidly and slowly adapting firing properties of primary afferents has been observed in different sensory modalities. For example, mechanosensitive neurons fall into two broad classes: those that fire throughout the duration of a stimulus (Merkel’s receptors and Ruffini’s corpuscles) and those that fire only transiently at the onset and offset of the stimulus (Pacinian and Meissner’s corpuscles). Furthermore, neural elements in addition to specialized receptors contribute to the rapidly adapting property (Loewenstein and Mendleson 1965). Moreover, a number of voltage-dependent ionic currents in dorsal root ganglion neurons was distributed according to size, suggesting that different electrophysiological properties characterize each submodality (McLean et al. 1988; Scroggs and Fox 1992; Scroggs et al. 1994). Retinal ganglion cells also can be separated into two major classes on the basis of adaptation to prolonged stimuli. In the cat retina, Y cells exhibit rapidly adapting firing properties, whereas X cells display slow adaptation to similar stimuli (Cleland et al. 1971; Shapley and Perry 1986). Therefore it is not unprecedented to detect these two firing patterns within a population of sensory neurons; for the primary auditory neurons, however, we do not yet know whether this endogenous feature corresponds to the type I/ type II classification.

Spiral ganglion neurons in each firing category did not possess uniform electrophysiological features. Instead, magnitudes or voltage dependence of interspike interval, spike amplitude decrement, and inward rectification differed from neuron to neuron. These graded features could clearly have an impact on signal transmission because action potential
et al. 1993), two different types of calcium-activated potassium neurons that were acutely isolated, without significant neurite outgrowth, displayed both rapidly and slowly adapting neurons (filled triangles) held at −80 mV.

Decline in spike amplitude was also observed in recordings of spontaneous activity from spiral ganglion neurons in vivo when they followed another action potential in close succession (<6 ms) (Siegel 1992). This firing characteristic was attributed to a potassium channel type with properties similar to those described by Hodgkin and Huxley (1952) in the squid giant axon. However, in addition to the multiple delayed rectifiers that could mediate this response (Davis 1996; Jonas et al. 1989; Safronov et al. 1993; Scholz et al. 1993), two different types of calcium-activated potassium currents, the BK (or maxi) \( I_{K(Ca)} \) and the SK (or slow) \( I_{K(Ca)} \), often underlie fast and slow afterhyperpolarizations, respectively (Hille 1992; Rudy 1988). Because of its voltage dependence and calcium activation, the BK \( I_{K(Ca)} \) classically mediates fast afterhyperpolarization and contributes to functions such as firing frequency regulation and spike repolarization. The SK \( I_{K(Ca)} \) generally displays very little voltage dependence and is activated predominately by Ca\(^{2+}\) accumulation; this channel type contributes to slow afterhyperpolarization, which is involved in regulating spike frequency adaptation (Hille 1992), such as that observed in the present study. Although there is no evidence that either the BK or SK \( I_{K(Ca)} \) channel types are present in chick or guinea pig spiral ganglion neurons (Santos-Sacchi 1993; Yamaguchi and Ohmori 1990), the BK \( I_{K(Ca)} \) has been localized to the internodal membrane of a select population of primary auditory neurons in goldfish (Davis 1996) and thus may also contribute to membrane repolarization in cells of the present study.

Additional experiments are required to define the pharmacological and voltage-dependent characteristics of the ionic currents that underlie the inward rectification we have observed; however, the time dependence of rectification and the associated rebound depolarization are features that are consistent with the \( I_{K} \) current (Mayer and Westbrook 1983; McCormick and Pape 1990; Nisenbaum and Wilson 1995; Spain et al. 1987). Originally characterized in cardiac Purkinje fibers (DiFrancesco 1981), this type of inward rectifier current is carried by both Na\(^{+}\) and K\(^{+}\) ions. Therefore the reversal potential of the \( I_{K} \) current is positive to the potassium reversal potential, suggesting that the function of this channel type is to maintain the resting potential close to firing threshold. Furthermore, there is a precedence for the nonuniform distribution of inward rectification that we have observed in spiral ganglion neurons. The \( I_{K} \) current has been shown to be differentially distributed among dorsal root ganglion cells (Mayer and Westbrook 1983), auditory thalamic neurons (Hu 1995), neostriatal neurons (Nisenbaum and Wilson 1995), and visual cortical projection neurons (Solomon et al. 1993).

Because the firing properties of neurons can change during development (for review see Spitzer 1991, 1994; Wong 1993), it is possible that neonatal spiral ganglion neurons have not yet developed their full complement of endogenous voltage-dependent channel types. Neurons were removed from animals soon after birth because they survive for days to weeks in vitro and display robust outgrowth. Nevertheless, the final refinements of cochlear ontogeny, such as synaptogenesis, have not yet been completed at this time (Walsh and Romand 1992). Therefore it is not known whether voltage-dependent channel types remain unaltered during these latter stages of development. Related to this issue is whether neurons display different firing patterns in response to local changes in culture conditions or due to differences in neurite outgrowth. Additional experiments on mature, acutely dissociated neurons will most likely resolve these uncertainties. Nevertheless, we have observed no systematic differences of \( AP_{\text{max}} \) and voltage dependence of \( AP_{\text{max}} \) when measurements were correlated to either the postnatal age (postnatal day 1–6), the amount of time in vitro (1–13 days), or the combination of these factors. Furthermore, marine neurons that were acutely isolated, without significant neurite outgrowth, displayed both rapidly and slowly adapting firing characteristics. In addition, a similar percentage of acutely isolated cells displayed high \( AP_{\text{max}} \) levels (11%; 2 of 18 neurons) compared with cells maintained for several days in vitro (unpublished data).

There are only a few studies of the endogenous electrical characteristics of spiral ganglion neurons (Santos-Sacchi 1993; Yamaguchi and Ohmori 1990). Although they focused on currents that underlie the neuronal firing patterns, these investigators did not find different electrophysiological classes or gradation of the recorded currents. One of these reports, however, was based exclusively on recordings from type I neurons (Santos-Sacchi 1993). Furthermore, as discussed above, age-related factors could account for time-dependent changes in neuronal firing, and two different de-
Developmental time points (embryonic and adult) were examined. The studies above also used spiral ganglion neurons from different species (chick and guinea pig). Preliminary work from our laboratory has shown species-specific properties in that gerbil neonatal spiral ganglion neurons fired fewer numbers of action potentials when tested under conditions identical to those of the present study; nevertheless, two electrophysiological classes were still evident in these cells. Other features, such as the graded nature of inward rectification, were also similar to those reported here (unpublished data).

Whether the neuronal firing characteristics that we observed pertain to auditory signal coding or developmental processes, the repercussions of the different firing categories are clear. Rapidly adapting neurons signal transitions in a stimulus, whereas slowly adapting neurons preserve information about stimulus duration and magnitude. Moreover, the graded nature of the inward rectification may lead to a continuum of firing thresholds for spiral ganglion neurons and could contribute to features such as the shape of the rate-intensity functions and spontaneous rate of firing. Regardless of the precise functional roles of the endogenous firing properties, spiral ganglion neurons do fall into electrophysiologically distinct classes that may be essential to the extraction of auditory information from the periphery.

We gratefully acknowledge Dr. Kewa Mou’s contributions to tissue culture preparation and immunohistochemistry. We appreciate Dr. Mark R. Plummer’s critical reading of an earlier version of the manuscript and thank two anonymous reviewers for helpful comments.

This research was supported by the Deafness Research Foundation and National Institute of Deafness and Other Communicative Disorders Grant R29 DC-01856.

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Received 11 July 1996; accepted in final form 18 November 1996.

REFERENCES


FIG. 10. Current-clamp recordings from a variety of holding potentials reveal hereogeneity in both magnitude and voltage dependence of inward rectification. A: difference between peak and steady-state voltage measurements plotted as a function of constant current injection from step potentials of −80 mV (●), −60 mV (○), and −40 mV (△). For this neuron there was only a small difference between the values measured at the more hyperpolarized membrane potentials; the greatest amount of inward rectification was detected at −40 mV. B: difference between peak and steady-state voltage measurements for a different neuron plotted as a function of constant current injection from membrane potentials of −100 mV (●), −80 mV (○), −60 mV (○), and −40 mV (△). For this cell inward rectification was minimal at −100 mV, was moderate at −80 mV, and reached maximal levels at −60 and −40 mV. The lack of inward rectification at more negative potentials suggests that the inward rectifier channels were fully open at those combinations of step and holding potentials. C: slope of a line fitted to the difference between peak and steady-state voltage measurements vs. current injection (shown in A) plotted as a function of holding potential for 5 different cells. All 5 examples are from rapidly adapting neurons that did not reach an identifiable maximum at −40 mV. D: slope of a line fitted to the difference between peak and steady-state voltage measurements vs. current injection (shown in B) plotted as a function of holding potential for 6 different neurons. All but 1 of the recordings were obtained from rapidly adapting neurons (gray diamonds). All 6 had similar voltage dependence, but different magnitudes of inward rectification.