Electrical Membrane Properties of Trapezoid Body Neurons in the Rat Auditory Brain Stem Are Preserved in Organotypic Slice Cultures

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ABSTRACT: The medial nucleus of the trapezoid body (MNTB) is a conspicuous structure in the mammalian auditory brain stem. It is a major component of the superior olivary complex and is involved in sound localization. Recently, organotypic slice culture preparations of the superior olivary complex were introduced to investigate the development of inhibitory and excitatory projections (Sanes and Hafidi, 1996; Lohmann et al., 1998). In the present article, we further assessed the organotypicity of our culture system (Lohmann et al., 1998) and examined electrical membrane properties of MNTB neurons expressed under culture conditions. To do so, MNTB neurons from early postnatal rats (P3–5) were studied after 3–6 days in vitro (DIV) by whole-cell patch-clamp recordings. Their mean resting potential was −59 mV, the input resistance averaged 171 MΩ, and the average time constant was 3 ms. Four types of voltage-activated conductances were observed in voltage-clamp recordings. All cells expressed a tetrodotoxin (TTX)-sensitive sodium current. Two types of potassium currents could be characterized: a tetraethylammonium (TEA)-sensitive and a 4-aminopyridine (4-AP)-sensitive conductance, both of which are composed of a transient and a sustained component. Finally, an inwardly rectifying current, activated by hyperpolarizing voltage steps, was found. In current-clamp recordings, depolarizing current pulses typically elicited a single action potential. In the presence of 4-AP, however, these current pulses induced a train of action potentials. The duration of action potentials was increased by 4-AP and the afterhyperpolarization was reduced. Hyperpolarizing current injections induced a “sag” in the membrane potential, indicating the influence of an inwardly rectifying current. Our results demonstrate that MNTB neurons in slice cultures have electrical membrane properties comparable to those of their counterparts in acute slices.

Keywords: auditory brain stem; organotypic slice culture; voltage-activated currents; rat

The superior olivary complex (SOC) is the first station in the mammalian auditory brain stem where input from both ears converges and binaural information is analyzed. Among other nuclei, the SOC includes the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO). MNTB neurons receive excitatory, glutamatergic input from the contralateral cochlear nucleus and convert this information to an inhibitory, glycinergic input to the ipsilateral LSO. LSO neurons also receive excitatory, glutamatergic inputs emerging from neurons in the ipsilateral cochlear nucleus. Thus, they compute intensity differences occurring at the two ears and are involved in the localization of sound sources (Irvine, 1986). The SOC nuclei show a precise topographical organization of both the excitatory and the inhibitory connections, which is why this brain area serves as a suitable system to study the development of these two types of connections (Sanes and Siverls, 1991; Kandler and Friauf, 1993, 1995a; Sanes, 1993).
Recently, a slice culture preparation of the rat SOC was introduced to investigate the mechanisms of synaptic development of the glycinergic projection between MNTB and LSO (Lohmann et al., 1998). Generally, slice cultures enable one to study the characteristics and the development of axonal connectivities within and between different brain areas, mainly because they permit experimental manipulations and are accessible to electrophysiological recording methods (Gähwiler, 1981; Llano et al., 1988; Knöpfel et al., 1990; Bolz et al., 1990; Stoppini et al., 1991). A prerequisite for the use of such slice cultures is their organotypic organization and the presence of cells displaying a high degree of cellular differentiation (Gähwiler et al., 1991). SOC cultures appear to reflect the in vivo situation, owing to the maintenance of many organotypic features such as the cytoarchitecture of the nuclei, specific internuclear connections, and the neuronal morphology (Lohmann et al., 1998). Interestingly, SOC nuclei in culture require an elevated extracellular KCl concentration to obtain organotypicity. The raised extracellular KCl obviously induces a membrane depolarization and, consequently, an influx of calcium ions via voltage-activated Ca\(^{2+}\) channels (Lohmann et al., 1998), indicating that an optimal intracellular Ca\(^{2+}\) concentration is necessary to maintain the viability of SOC neurons, as was also shown for other types of neurons (Hegarty et al., 1997; Abdel-Hamid and Tymianski, 1997).

Hitherto, nothing has been known about the electrical membrane properties of SOC neurons in slice cultures. Particularly with respect to the culture conditions, i.e., the chronically raised KCl concentration, which probably causes a sustained depolarization of the cells lasting throughout the entire culture period, it is important to know whether the neurons exhibit normal electrical membrane properties such as voltage-activated conductances. Electrical membrane properties constitute the basis for several physiological parameters, e.g., resting potential, firing threshold, and firing rate. Thus, they participate in the transmission of information between neurons.

The aim of the present study was to investigate the electrical membrane properties expressed by MNTB neurons under culture conditions, to assess whether, aside from the anatomical features, physiological features are also preserved in our culture system. To do so, whole-cell patch-clamp recordings were performed in the voltage-clamp and current-clamp mode. MNTB neurons were chosen for two reasons: (a) Their membrane properties have been described extensively in acutely prepared slices (Wu and Kelly, 1991; Banks and Smith, 1992; Banks et al., 1993; Forsythe and Barnes-Davies, 1993; Brew and Forsythe, 1995) (Table 1), thus allowing us to compare their characteristics with those of their counterparts in vitro; and (b) it can be assumed that cultured MNTB neurons need to maintain certain electrical membrane properties to provide adequate inhibitory input to the LSO, whose anatomy is well preserved in vitro (Lohmann et al., 1998). Some of the results have been communicated elsewhere in abstract form (Löhrke et al., 1998).

**MATERIALS AND METHODS**

Slice Culture

The preparation of slice cultures was based on the method of Stoppini et al. (1991) and was described in detail elsewhere (Lohmann et al., 1998). Briefly, young Sprague–Dawley rat pups [postnatal day (P) 3–5] were killed by an overdose of ketamine (1 g kg\(^{-1}\)) and decapitated, and the brain was carefully dissected under sterile conditions. The following preparation steps were done in ice-cold, modified artificial cerebrospinal fluid (ACSF) (for composition, see below): Coronal brain stem slices containing the SOC were cut (300 \(\mu\)m) with a vibrating microtome (Vibracut 3; FTB, Bensheim, Germany; or VT 1000 S, Leica, Bensheim, Germany) and placed on CM membranes (Millipore, Bedford, MA) in six-well culture plates (Becton Dickinson, Heidelberg, Germany). Following the addition of 1 mL culture medium to each well, the slices were incubated for 3–6 days at 36.5°C in a humified atmosphere containing 5% CO\(_2\). The culture medium was changed three times a week.

Electrophysiological Recordings

Whole-cell patch-clamp recordings were performed on 74 MNTB neurons after 3–6 days in vitro (DIV). First, slices were transferred from the CM membranes into a chamber containing carboxygenated (95% O\(_2\), 5% CO\(_2\)) extracellular solution for 30 min at room temperature (20–23°C). Thereafter, they were placed in a continuously perfused recording chamber (perfusion rate ≈ 2.5 mL min\(^{-1}\)) which was mounted on an upright microscope (Axioskop FS; Zeiss, Jena, Germany). The microscope was equipped with differential interference contrast optics and an infrared video camera system (Hamamatsu, Herrsching, Germany) to improve visual identification of neurons within the slice. Patch pipettes were pulled from borosilicate glass capillaries (GB 150F-8P; Science Products, Hofheim, Germany) with a vertical puller (PP-83; Narishige, London, UK). They had resistances of 5–7 MΩ and were connected via an Ag/AgCl wire to an...
Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Series resistances were compensated for 72–80% by the circuit of the amplifier. Because of the large current amplitudes of MNTB neurons obtained in voltage-clamp experiments (up to 10 nA), command potentials \( V_C \) were corrected for voltage errors caused by the remaining series resistance \( V_R \) and are given as membrane potentials \( V_M = V_C - V_R \) when calculating current–voltage relations. Membrane potentials measured in current-clamp mode are indicated as \( V_M \). Liquid junction potentials were calculated using the program JPCalc by Barry (1994); as they amounted to -3.3 mV, they were not corrected. In voltage-clamp experiments with depolarizing command potentials, leak currents were determined by a P/4 protocol and subtracted on-line from voltage-activated currents. Voltage-clamp recordings with hyperpolarizing command potentials were performed without leak subtraction. Analog signals were low-pass filtered at 5 or 10 kHz, digitized, and sampled at a frequency of 14 or 20 kHz. Data were acquired and analyzed with commercial software (pCLAMP 6.0.3, Axon Instruments; and ORIGIN 3.5, Microcal, Northampton, MA). Data were tested for normal distribution and are given as the average value ± standard deviation. Statistical differences between age groups were tested using linear regression analysis.

**Solutions and Drugs**

The ACSF used for preparation contained \( (mM) \): KCl 2.5, MgCl₂ 1, CaCl₂ 2, d-glucose 260, NaHCO₃ 26, NaH₂PO₄ 1.25, Na-pyruvate 2, myo-inositol 3, and kynurenic acid 1 (pH 7.4). Culture medium consisted of 24% horse serum, 48% minimum essential medium, 24% Earl’s balanced salt solution, 1% glutamine (200 mM), 2.75% glucose solution (200 g L⁻¹), and 25 mM KCl. Except for KCl, all compounds were obtained from Life Technologies (Eggenstein, Germany). The extracellular solution had the following composition \( (mM) \): NaCl 125, KCl 2.5, NaHCO₃ 26, NaH₂PO₄ 1.25, MgCl₂ 1, CaCl₂ 2, d-glucose 10, Na-pyruvate 2, myo-inositol 3 (pH 7.4). Patch pipettes were filled with a solution containing \( (mM) \): KCl 130, EGTA 5, Hepes 10, MgCl₂ 1, Mg₂ATP 2, and Mg₂GTP 0.3 (pH 7.2). Drugs were dissolved in the extracellular solution and bath-applied by switching between different inflow channels. The used drugs were obtained from the following suppliers: tetrodotoxin (TTX), Biotrend (Cologne, Germany); tetraethylammonium chloride (TEA), Fluka (Buchs, Switzerland); and 4-aminopyridine (4-AP), Sigma (Deisenhofen, Germany).

**RESULTS**

**Cell Identification and Passive Membrane Properties**

Because of the preserved cytoarchitecture of the nuclei and the neuronal morphologies in SOC slices cultured with 25 mM KCl (cf. Lohmann et al., 1998), MNTB neurons could be easily identified by direct observation [Fig. 1(A)] (compare with Fig. 1 in Forsythe and Barnes-Davies, 1993; and Barnes-Davies and Forsythe, 1995, which depict the appearance seen in acute slices). Figure 1(B) shows the effect of elevated extracellular KCl (25 mM) on the membrane potential \( V_M \) of an MNTB neuron recorded in current-clamp mode. As expected from the Goldman equation, the neuron was depolarized by approximately 35 mV, reaching a steady-state potential at about -40 mV which returned to the resting potential during the washout of 25 mM KCl. This result indicates that a tonic
Figure 1 MNTB neurons in organotypic slice cultures. (A) High-magnification photomicrograph of a slice culture placed in the recording chamber after 4 DIV, showing axons (arrows) and a high density of oval-shaped cell bodies in the MNTB. Scale bar = 20 μm. (B) Example of a current-clamp recording in which application of 25 mM KCl (indicated by the solid bar) induced a tonic, reversible membrane depolarization of approximately 35 mV. The cell illustrated is one of nine neurons which had a resting potential \( V_R \) \( \approx \) -70 mV. The long latency of the response was due to the slow bath application system.

Depolarization of similar amplitude is likely to be present during the entire culture period of several days, during which 25 mM KCl is continuously applied. Based on this result, the electrical membrane properties of 74 MNTB neurons were analyzed in slice cultures adapted to normal extracellular solution for at least 30 min (see Methods).

The resting potential \( V_R \) was determined immediately after the establishment of the whole-cell configuration. For all MNTB neurons, irrespective of the culture duration or the age when slices were prepared, \( V_R \) averaged \(-59 \pm 8\) mV (\( n = 69 \); range \(-50 \) to \(-77\) mV). To elucidate potential developmental changes of \( V_R \) during the observed time period, \( V_R \) was analyzed in more detail as illustrated in Figure 2. Neurons were categorized into three groups according to the age when slices were prepared (P3, P4, and P5) and their \( V_R \) were plotted against the days \( \text{in vitro} \) (3–6 DIV). None of the three groups showed significant correlation between \( V_R \) and the culture duration \( \{P3 \ (p = .09); P4 \ (p = .24); P5 \ (p = .67)\} \), indicating no developmental change of \( V_R \) between 3 and 6 DIV. The mean \( V_R \) of MNTB neurons from P3, P4, and P5 slice cultures amounted to \(-57 \pm 5\) mV (\( n = 8 \)), \(-61 \pm 8\) mV (\( n = 38 \)), and \(-57 \pm 6\) mV (\( n = 23 \)), respectively. In addition, the population of neurons kept for 4 DIV (\( n = 36 \)) showed no significant correlation (\( p = .63 \)) between \( V_R \) and the time of preparation, indicating no developmental change of this parameter between P3 and P5. Together, these results indicate that \( V_R \) remained unchanged during the observed time period, both \( \text{in vivo} \) (P3–5) and \( \text{in vitro} \) (3–6 DIV). This is in accordance with the finding that neurons in acute SOC slices, including the population of MNTB neurons, showed no significant change in passive membrane properties during development between embryonic day (E) 18 and P17.

Figure 2 Analysis of the resting potential \( V_R \) of MNTB neurons in organotypic slice cultures. Neurons were categorized into three groups according to the postnatal age when slices were prepared (P3, P4, and P5); moreover, their resting potentials are plotted against the days \( \text{in vitro} \) (3–6 DIV). None of the three groups showed significant correlation between \( V_R \) and culture duration (see text for details). \( n = \) number of tested neurons; \( r = \) correlation coefficient.
Membrane Properties of Auditory Brain Stem Neurons in Culture (Kandler and Friauf, 1995b). With respect to this, we investigated other passive membrane properties, such as input resistance and time constant, irrespective of when slices were prepared or how long the neurons were kept in culture. The input resistance was determined from the linear part of the current-voltage relation in current-clamp recordings [see also Δ in Fig. 8(C,D)] and averaged 171 ± 56 MΩ ($n = 14$). The time constant was determined by a single exponential fit from voltage traces induced by small hyperpolarizing or depolarizing 400-ms current pulses which did not affect voltage-activated conductances; it averaged 3 ± 2 ms ($n = 14$). Input resistance and time constant both showed no significant change during the observed time period ($14$ pooled neurons; $p = .45$ and $p = .54$, respectively).

Because the analysis of the dynamic changes of active membrane properties of MNTB neurons in slice cultures was not the aim of this study, voltage-activated membrane conductances will be described mainly qualitatively in the following, regardless of the postnatal age and/or the duration of the culture.

**Sodium Currents**

All 74 MNTB neurons showed rapidly activating inward currents, followed by outward currents, in response to depolarizing voltage steps, which started from a holding potential ($V_H$) of -70 mV. An example of this result is illustrated in Figure 3(A). The inward current was blocked by the specific Na⁺ channel antagonist TTX. When 0.5 μM TTX was used, the blockade averaged 90 ± 17% ($n = 17$) [Fig. 3(B)]; when 2 μM TTX was applied, the blockade was complete ($n = 6$ of 6).

**Figure 3** Analysis of the TTX effect on depolarization-induced currents. (A) Superimposed current traces in response to a family of depolarizing voltage steps with a duration of 40 ms. At command potentials positive to -40 mV, a fast inward component was elicited (downward deflection), followed by a tonic outward component (upward deflection). (B) Bath application of TTX completely blocked the fast inward current, but it also decreased the outward current. (C) Digital subtraction of the current traces from (A) and (B) at an expanded time scale, to visualize the current components affected by TTX. $V_H$ = holding potential; $V_C$ = command potential protocol: first voltage step/last voltage step; voltage increment (the same information about stimulus protocol is provided in Figs. 4, 5, and 8).
increase of the series resistance occurring during the course of the experiments or an influence of Na⁺ on outward currents, such as previously described for heart ventricle cells of dogs (Zygmunt et al., 1997).

From Figure 3(C), it is also apparent that the activation kinetics of fast voltage-activated currents occasionally showed voltage-dependent variability, obviously due to an insufficient voltage-clamp of the morphologically complex MNTB neurons (for details of cell morphology in culture, see Lohmann et al., 1998). Because of this finding, we did not analyze further the fast inward current. Likewise, we did not quantify the kinetics of other voltage-activated conductances that will be described in the following.

**Potassium Currents**

Pharmacological properties of outward currents were tested on nine MNTB neurons which were voltage-clamped at $V_h = -70$ mV and continuously perfused with extracellular solution containing 0.5 μM TTX. Outward currents were induced by 450-ms voltage steps between $-60$ and 20 mV, in 10-mV increments. The effects of the two potassium channel antagonists TEA and 4-AP, used at concentrations of 10 and 0.2 mM, respectively, were tested.

Tetraethylammonium chloride, which is known to block a delayed rectifier potassium current (Hille, 1992), reduced the amplitude of the outward current in all neurons tested ($n = 5$) [Fig. 4(A,B) and Table 1]. To reveal the TEA-sensitive conductance, the current traces obtained in the presence of TEA were digitally subtracted from those obtained under control conditions [Fig. 4(C)]. Consequently, the lower trace in Figure 4(C) shows the TEA-sensitive current in response to a voltage step from $-70$ to 20 mV, illustrating that the current was composed of a transient and a sustained component.

A quantitative analysis of the TEA-sensitive conductance was carried out on five cells by current–voltage (I-V) relations based on $V_m$ values ($V_m = V_c - V_h$) (see Methods); only the sustained current component was analyzed. To do so, current amplitudes were measured at the end of the responses, as indicated by triangles in Figure 4(A,B). Currents obtained under control conditions ($\triangle$) or in the presence of TEA (▲) could be activated with command potentials positive to $-60$ mV [Fig. 4(D)]. The amount of the TEA-sensitive current was calculated by subtracting the integrals under the two I-V curves in the $V_m$ range between $-60$ and 0 mV [gray areas in Fig. 4(D)]. It corresponded to an average of $54 \pm 19\%$ ($n = 5$) of the total outward current.

Figure 5 shows the typical effect of 4-AP on the potassium outward current. The outward current was noticeably reduced by bath application of 0.2 mM 4-AP [Fig. 5(A,B)], and the blockade affected a current composed of a transient and a sustained component [see resulting curve after digital current trace subtraction in Fig. 5(C)]. Currents obtained under control conditions (○) or in the presence of 4-AP (●) could be activated at potentials positive to $-60$ and $-50$ mV, respectively [Fig. 5(D)]. The sustained 4-AP–sensitive component, obtained in the $V_m$ range between $-60$ and 0 mV, was calculated in the same way as for the TEA-sensitive component and amounted to $65 \pm 11\%$ ($n = 4$). Together, the results from the TEA and the 4-AP experiments (54% and 65% sensitivity, respectively) indicate some cross reaction of the two potassium channel blockers on the sustained outward current.

**Current-Clamp Measurements and Effects of 4-AP**

Several studies on acutely isolated brain stem slices have shown that MNTB neurons characteristically generate a single action potential in response to depolarizing current steps (Wu and Kelly, 1991; Banks and Smith, 1992; Forsythe and Barns-Davies, 1993; Brew and Forsythe, 1995). We tested a total of 57 cultured MNTB neurons held at $V_h$ and slightly negative to $V_h$ [indicated as pipette potential ($V_{pip}$)], by applying depolarizing current step families (step duration of 400 ms). About 81% of the neurons ($n = 46$ of 57) responded to depolarizing current injections exclusively with a single action potential at the start of the current steps, as illustrated in Figure 6(A1,C1) and Figure 8(C).

Figure 6 represents the effect of 4-AP on action potential firing observed in six neurons. In normal extracellular solution, depolarizing current pulses elicited a single action potential right after the start of the step [Fig. 6(A1)]. In the presence of 0.2 mM 4-AP, however, the same current step elicited a train of action potentials [Fig. 6(A2)]. The pattern of single action potential firing recovered several minutes after returning to normal extracellular solution [Fig. 6(A3)]. In the presence of 4-AP, the number of action potentials depended on the current amplitude [▲ in Fig. 6(B)]. In contrast, a maximum of one single action potential was induced under control conditions, irrespective of the current
Analysis of the TEA-sensitive current. The bath solution contained 0.5 μM TTX to suppress sodium currents. (A,B) Outward currents in response to depolarizing voltage steps were reduced in the presence of 10 mM TEA (B). (C) The two upper traces show the currents which were induced by a voltage step from −70 to 20 mV [cf. (A) and (B)] at an expanded time scale. The lower trace represents a digital subtraction of the two upper traces and depicts the TEA-sensitive outward current, which consists of a transient and a sustained component. (D) Current–voltage (I-V) relations of the sustained outward current component determined at the end of the 450-ms voltage step [△ in (A), ▲ in (B)]. The relative contribution of the TEA-sensitive current to the total outward current was determined in the $V_m$ range between −60 and 0 mV. It was calculated by subtraction of the integrals under the two I-V curves (indicated by the gray areas) and corresponded to 48%. Small upward and downward deflections at the start of the current traces correspond to residual capacitive artifacts and unblocked inward currents, respectively.

Additional 4-AP effects are illustrated in Figure 6(C) for another neuron and at an expanded time scale. 4-AP resulted in an increase of both the input resistance and the width of the action potential at half-amplitude and also in a reduction of the afterhyperpolarization am-
Figure 5  Analysis of the 4-AP–sensitive current. The sampling and analysis protocols were the same as in Figure 4, but 0.2 mM 4-AP instead of TEA was added to the bath solution. (A) and (B) show the reduction of the outward current amplitude caused by 4-AP. (C) The 4-AP–sensitive current (lower trace) was isolated by digital subtraction of the current seen in the presence of 4-AP from the control current (two upper traces). It consisted of a transient and a sustained component. (D) I-V relations of the outward currents from (A) (○) and (B) (●), each time measured at the end of a 450-ms-long voltage step. In the $V_m$ range between −60 and 0 mV, the 4-AP–sensitive current amounted to 62%. All data were recorded from the same neuron and in the presence of 0.5 μM TTX.
Figure 6 Analysis of the 4-AP effect on action potential firing recorded in current-clamp mode. Under control conditions (A1), a depolarizing current step of 0.41 nA elicited a single action potential. In the presence of 4-AP (A2), however, the same current step evoked a train of action potentials. (A3) This effect completely recovered after washout of 4-AP. (B) With increasing stimulus amplitude, a maximum of a single action potential was induced under control conditions [○; same cell as in (A)]. In contrast, the number of action potentials increased with current amplitude in the presence of 4-AP (▲). (C) Influence of 4-AP on action potential properties illustrated for another MNTB neuron. (C1) The single action potential elicited under control conditions had a width at half-amplitude of 1.7 ms and was followed by an afterhyperpolarization (arrow). (C2) 4-AP increased the width at half-amplitude of the second and third action potential to 6.2 and 7.0 ms, respectively; it also reduced the amplitude of the afterhyperpolarization (arrows). Bottom trace in (A) and (C) indicates duration and amplitude of the injected current pulse. Note the differences in time scales in (A) and (C).
We found seven neurons which fired a single action potential with small current injections [Fig. 7(A1)], whereas they generated a series of action potentials at larger current injections [Fig. 7(A2)]. Another four cells showed a series of action potentials, irrespective of the amplitude of the depolarizing current steps [Fig. 7(B1,2)]. Six of the above-mentioned 11 cells were tested to assess whether they exhibited spontaneous activity which could affect the firing behavior in response to depolarizing current injections. Indeed, depending on the membrane potential, all six neurons showed either spontaneous action potentials [trace 3 in Fig. 7(A,B)] or spontaneous postsynaptic potentials [arrows in trace 4 of Fig. 7(A,B)].

Because these six cells could not be properly evaluated, we excluded them from the sample. By doing so, the proportion of MNTB neurons generating a characteristic single action potential firing behavior increased to 90% (n = 46 of 51) (Table 1).

**Inward Rectification**

Aside from the 4-AP-sensitive conductance, MNTB neurons in acute slices display a further prominent voltage-dependent current, i.e., a hyperpolarization-activated, mixed cation current described as $I_h$ (Banks et al., 1993; Forsythe and Barnes-Davies, 1993). To analyze the presence of $I_h$ on cultured MNTB neurons, we tested a total of 41 neurons by applying hyperpolarizing voltage steps between $-70$ and $-150$ mV, starting from $V_h = -60$ mV with $-10$-mV increments (step duration between 750 ms and 2.5 s; recorded without leak subtraction). The majority of cells (n = 37 of 41) (Table 1) responded with an inward current which was reminiscent of $I_h$. An example for such a current is shown in Figure 8(A). At potentials more negative than $-70$ to $-80$ mV, a slowly activating inward current occurred. Only four neurons displayed exclusively an instantaneous inward current. Figure 8(B) shows the I-V relations of the instantaneous ($\square$) and the steady-state ($\blacksquare$) currents illustrated in Figure 8(A). The open circles correspond to the slowly activating inward current as determined by the digital subtraction of these two currents. When neurons exhibiting a slowly activating inward current were tested in current-clamp mode (n = 5), an inward rectification behavior was obvious in response to the hyperpolarizing stimulus, which appeared as a sag in the membrane potential [Fig. 8(C)]. In Figure 8(D), the voltage differences ($V_{Mem} - V_h$), as they occurred at the beginning ($\Delta$) and the end ($\triangle$) of the current steps, are plotted against the injected current amplitude [see corresponding symbols in Fig. 8(C)]. Inward rectification behavior occurred at potentials approximately 20 mV more negative than the resting potential [$\blacktriangle$ in Fig. 8(D)]. This behavior was in accordance with the activation of $I_h$ measured in the voltage-clamp mode [Fig. 8(A,C); same cell].

**DISCUSSION**

The present report represents the first analysis of the electrophysiological properties of auditory brainstem neurons kept in a slice culture preparation. Our results demonstrate that cultured MNTB neurons exhibit a considerable number of normal passive and active electrical membrane properties. Our results thus indicate that the culture conditions used here (i.e., the high extracellular potassium concentration) do not influence the cell-specific expression of voltage-activated conductances as well as the membrane properties responsible for the neurons’ resting potential.

**Passive Membrane Properties**

After adaptation to normal extracellular KCl concentration for more than 30 min, MNTB neurons in slice cultures had resting potentials between $-50$ and $-77$ mV. These values comply with those obtained from MNTB neurons in acutely isolated slices with intracellular recordings ($-62$ mV) (Banks and Smith, 1992) or patch-clamp recordings ($-70$ mV) (Forsythe and Barnes-Davies, 1993). All values appear to reflect the condition of intact neurons.

In acute slices, different values have been obtained for the input resistance of MNTB neurons (44 MΩ, Banks and Smith, 1992; 250 MΩ, Forsythe and Barnes-Davies, 1993), which was obviously due to the different recording techniques used: The lower value, obtained with sharp intracellular electrodes, possibly reflects some penetration damage. MNTB neurons in slice cultures had a slightly smaller input resistance than those in acutely isolated slices (mean 171 vs. 250 MΩ) (Forsythe and Barnes-Davies, 1993) despite the fact that both studies employed patch-clamp recordings. It remains unclear whether the difference is statistically significant.

The membrane time constant was considerably smaller in cultured MNTB neurons compared to...
Figure 7 Spontaneous activity occurring in cultured MNTB neurons. (A) Example of a neuron which fired a single action potential in response to a 0.17-nA current pulse (A1), yet a series of action potentials at a 0.42-nA current pulse (A2). The same cell showed spontaneous
MNTB neurons in acute slices (mean 3 vs. ~ 14 ms) (Forsythe and Barnes-Davies, 1993). This is probably caused by the differences in input resistance, although no information is available about the membrane capacities under the different conditions. Furthermore, it is conceivable that the discrepancy is due to the different pulse protocols used: To determine the time constant, we applied depolarizing or hyperpolarizing current pulses of 400-ms duration as opposed to 1-ms hyperpolarizing current pulses used by Forsythe and Barnes-Davies (1993).

**Voltage-Activated Currents**

Whole-cell patch-clamp studies of voltage-activated conductances in morphologically complex cells are often restricted by technical problems, such as the lack of voltage control in cell regions far away from the recording electrode, resulting in an incomplete voltage clamp. Consequently, we did not describe the kinetics of any voltage-activated current. Nevertheless, we are convinced that our qualitative description of voltage-activated currents in MNTB neurons in slice cultures allows a comparison with the data obtained from their counterparts in acute slices.

The existence of a TTX-sensitive sodium current in MNTB neurons of acutely isolated slices was demonstrated by Forsythe and Barnes-Davies (1993). In their study, a detailed analysis of this rapidly activating sodium conductance was not done owing to an incomplete voltage clamp of the cells. Interestingly, similar to our observation, the application of TTX resulted in both the block of the inward sodium current and the reduction of an outward current, which can be deduced from the inset of Figure 2(B) in Forsythe and Barnes-Davies (1993). The reason for the observed reduction of the outward current during TTX application is unclear. To elucidate if sodium ions indeed influence the outward currents in MNTB neurons, a detailed analysis, following the protocols by Zygmunt et al. (1997) would be necessary.

In contrast to the somehow neglected TTX-sensitive sodium current, different cation currents have...
Figure 8 Analysis of inward rectification behavior in voltage-clamp and current-clamp recordings. (A) Current traces in response to a family of 750-ms-long hyperpolarizing voltage steps. Upward and downward deflections at the beginning and end of the current traces correspond to capacitive artifacts. (B) Diagram of the I-V relations of the instantaneous (□) and the steady-state current (■) derived from the current traces in (A). The I-V relation of the slowly activating inward current (○) was calculated by subtraction of the two other values. (C) Voltage traces in response to a family of 400-ms-long current steps (I_inj), starting from the cell’s resting potential (V_R). During the hyperpolarizing stimuli, inward rectification behavior appeared as a sag in the membrane potential. (D) Current-clamp I-V relations of the transient (▲) and the steady-state responses (△), determined from the voltage traces in (C) at time points indicated by the corresponding symbols.

(Banks et al., 1993). Like the other above-mentioned conductances, I_h belongs to the typical set of voltage-activated currents in MNTB neurons, which are also preserved under culture conditions. Comparable to the situation in slice cultures, some MNTB neurons in acutely isolated slices possess only an
instantaneous inward current component in response to hyperpolarizing voltage steps (Forsythe and Barnes-Davies, 1993).

Pattern of Action Potential Firing

In our slice cultures, the majority of MNTB neurons typically responded with a single action potential at the start of depolarizing current steps, comparable to MNTB neurons in acutely isolated slices (Wu and Kelly, 1991; Banks and Smith, 1992; Forsythe and Barnes-Davies, 1993; Banks et al., 1993; Brew and Forsythe, 1995). This response could be attributed to a distinct set of voltage-activated conductances, mainly the 4-AP-sensitive potassium current. The unusual response pattern of repetitive action potentials, which occurred in about 19% (11 of 57) of the cultured MNTB neurons, was probably due to spontaneous action potential activity which occurred during the application of current injections. Banks and Smith (1992) described two of 47 MNTB neurons in acutely isolated brain stem slices as “non-principal” cells because of their different morphology and physiological characteristics. Non-principal cells fire sustained trains of action potentials in response to depolarizations and exhibit a slight sag in the membrane potential in response to hyperpolarizations. Although we recorded from cells which we visually identified as principal MNTB neurons owing to their location and soma shape, we cannot exclude that some of the cells which exhibited repetitive action potentials were in fact non-principal MNTB neurons.

In summary, our results show that MNTB neurons in slice cultures express a set of voltage-activated conductances which is very similar to that described for MNTB neurons in acutely isolated brain stem slices. Therefore, they confirm the organotypicity of our slice culture. With respect to the role of voltage-activated conductances in processing and transmitting information, the conditions for further study of activity-dependent processes of synaptic development in slice cultures have been established. In this respect, it is remarkable that physiological properties of the neurons remain organotypic despite the fact that during the culture period, when 25 mM KCl is chronically applied, the neurons are possibly constantly depolarized to about −40 mV. Even if some voltage-activated conductances such as the sodium current and various potassium currents are inactivated under this condition (see also Brew and Forsythe, 1995), this does not influence the cell-specific expression of voltage-activated conductances. Future studies should address synaptic neurotransmission and the effects of synaptic blockade on the maturation of the microcircuitry in the SOC.

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