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Inhibitory and Excitatory Postsynaptic Currents of Medial Vestibular Nucleus Neurons of Rats

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The medial vestibular nucleus (MVN) neurons are controlled by excitatory synaptic transmission from the vestibular afferent and commissural projections, and by inhibitory transmission from interneurons. Spontaneous synaptic currents of MVN neurons were studied using whole cell patch clamp recording in slices prepared from 13- to 17-day-old rats. The spontaneous inhibitory postsynaptic currents (sIPSCs) were significantly reduced by the $GABA_A$ antagonist bicuculline (20 μM), but were not affected by the glycine antagonist strychnine (1 μM). The frequency, amplitude, and decay time constant of sIPSCs were 4.3±0.9 Hz, 18.1±2.0 pA, and 8.9±0.4 ms, respectively. Spontaneous excitatory postsynaptic currents (sEPSCs) were mediated by non-NMDA and NMDA receptors. The specific AMPA receptor antagonist GYKI-52466 (50 μM) completely blocked the non-NMDA mediated sEPSCs, indicating that they are mediated by an AMPA-preferring receptor. The AMPA mediated sEPSCs were characterized by low frequency (1.5±0.4 Hz), small amplitude (13.9±1.9 pA), and rapid decay kinetics (2.8±0.2 ms). The majority (15/21) displayed linear I-V relationships, suggesting the presence of GluR2-containing AMPA receptors. Only 35% of recorded MVN neurons showed NMDA mediated currents, which were characterized by small amplitude and low frequency. These results suggest that the MVN neurons receive excitatory inputs mediated by AMPA, but not kainate, and NMDA receptors, and inhibitory transmission mediated by $GABA_A$ receptors in neonatal rats.

Key Words: Inhibitory postsynaptic current, Excitatory postsynaptic current, GluR2 AMPA receptor, $GABA_A$ receptor, Medial vestibular nucleus

INTRODUCTION

The medial vestibular nucleus (MVN) is involved in the reflex control of the head and eyes, and the recovery of vestibular function after vestibular lesions. MVN neurons are controlled by glutamatergic excitatory transmission from the vestibular afferent nerve terminal and by inhibitory transmission of the contralateral side via glutamatergic commissural projections connected to inhibitory interneurons (De Waele et al, 1995; Vidal et al, 1999).

Ionotropic glutamate receptors are subdivided into AMPA, kainite (KA), and NMDA receptors. AMPA receptor subunits are the products of four different genes, GluR1 to GluR4, kainate (KA) receptor subunits are the products of five different genes, GluR5 to GluR7 and KA1/KA2, and NMDA receptor subunits are classified into NMDAR1 (NR1) and NR2A-D. Physiological and pharmacological data indicate that NMDA and AMPA receptors play important roles in the mediation of glutamate neurotransmission and synaptic plasticity in the vestibular nuclei (De Waele et al, 1995; Vidal et al, 1999).

Recently, several drugs have been developed that have different effects on AMPA- and KA-preferring receptors. GYKI-52466 is a 2, 3 benzodiazepine that acts as a selective antagonist for AMPA receptors, but not for KA receptors (Doneran & Rogawski, 1993; Parsons et al, 1994). Cyclothiazide enhances the excitatory responses (mainly by blocking desensitization) mediated by AMPA-preferring receptors (Partin et al, 1993; Jacoby & Samuel, 2001). A previous study showed that MVN neurons respond in an excitatory fashion to bath-applied AMPA, kainate, and NMDA (Sakai et al, 1996). However, it is unclear whether both AMPA and kainate receptors are present on MVN cells, since kainate effectively activates both types of receptor (Hollmann & Heinemann, 1994). Recent in situ hybridization and immunocytochemical studies have shown that AMPA subunits (GluR1, GluR2, GluR2/3, and GluR4 subunits) are expressed in the MVN (Popper et al, 1997). Of the different AMPA receptor subunits, GluR2 plays a particularly important role in determining channel properties. AMPA receptors containing this subunit have very low permeability for Ca^{2+} ions, and a linear or outwardly rectifying I-V relationship (Geiger et al, 1995; Jonas & Burnashev, 1995). Conversely, receptors lacking GluR2

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ABBREVIATIONS: MVN, medial vestibular nucleus; sIPSCs, spontaneous inhibitory postsynaptic currents; sEPSCs, spontaneous excitatory postsynaptic currents; NMDA, N-Methyl-D-aspartic acid; AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

display high Ca^{2+} permeability and a characteristic inwardly rectifying I-V relationship (Bowie & Mayer, 1995; Lui & Cull-candy, 2002). Since Ca^{2+} permeability is important in synaptic plasticity, we analyzed the I-V relationship to confirm whether the GluR2 subunit is present.

There is substantial evidence that GABA and glycine mediate synaptic transmission in the MVN. Previous studies have demonstrated that vestibular neurons are endowed with $GABA_A$, $GABA_B$, and glycinergic receptors (Holstein et al, 1992; Vidal et al, 1999). About 30% of the MVN neuron population releases GABA. These cells correspond to the inhibitory interneurons and second-order neurons projecting to extraocular and spinal motoneurons (De Waele et al, 1995; Vidal et al, 1999). In view of the pivotal role of glutamatergic and GABAergic synapses on MVN neurons, we characterized their spontaneous excitatory and inhibitory synaptic transmission and investigated their functional properties using whole cell recording in brain slice preparations.

METHODS

Slice preparation

Thirteen- to 17-day-old Sprague-Dawley rats were anesthetized with ether and decapitated. The brainstem was rapidly dissected and submerged in 4°C artificial cerebrospinal fluid (ACSF). Slices of brainstem were prepared 200 μm thick coronally with a vibroslicer (752M, Campden Instruments, UK). These slices were then incubated in ACSF saturated with 95% O_2 – 5% CO_2 at 32°C for 1 h and transferred to a recording chamber (0.8 ml), which was continuously perfused with aerated ACSF at a rate of 1 ml/min.

Solution

The ionic composition of the ACSF (in mM) was 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 10 glucose, and 24 $NaHCO_3$ (pH 7.4). The pipettes were filled with a solution containing (in mM) 140 KCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 5 ethylene glycol-bis (-amino-ethylether)-N,N,N',N'- tetra-acetic acid (EGTA), 10 N-2-hydroxyethylpiperazine- N'-2-ethanesulfonic acid (HEPES), and 2 MgATP (pH 7.25). To study the agonist-evoked current, the KCl was replaced with equimolar CsCl, and QX-314 (4~5 mM) was included in the pipette solution.

Electrophysiological recording

Patch pipettes were pulled from borosilicate glass tubing (1.5 mm diameter, 0.25 mm wall thickness). When filled with internal solution, they had a resistance of 3~4 MΩ. MVN neurons were identified using an upright microscope equipped with Nomarski optics (×400, BX50WI, Olympus, Japan). Membrane currents were recorded in the whole cell configuration of the patch clamp technique using an Axopatch 200B amplifier (Axon Instruments, USA). Series resistance was compensated by about 80% using the internal compensation circuits of the amplifier. After filtration at 2 KHz using a low-pass filter, data were acquired using a Digidata 1200 interface and pClamp software (version 8.0, Axon Instruments, USA) for subsequent analysis.

Analysis of synaptic currents

The spontaneous postsynaptic currents (sPSCs) were analyzed using the Mini Analysis Program (version 5.0, Synaptosoft, USA). The frequency of sPSCs was determined by setting a detection threshold level above the current noise. Mean amplitudes and frequencies of sPSCs were computed from all sPSCs observed in an MVN neuron. The numbers of exponentials necessary for a good fit of the decay time constant were determined by visual inspection. All data are given as means±S.E.M. The statistical significance of the data was determined using Students unpaired t-test to compare two means. A significance level of $p<0.05$ was considered significant.

RESULTS

In 27 of 32 MVN neurons, sPSCs were observed in the presence of the excitatory amino acid antagonists DNQX (20 μM) and AP5 (50 μM) at a holding potential of −60 mV. These spontaneous currents were completely and reversibly blocked by bicuculline (20 μM), but were not affected by strychnine (1 μM), indicating that they were mediated by $GABA_A$ receptors (Fig. 1). The reversal potential of sIPSCs was close to the Cl^- equilibrium potential (0 mV) using 140 mM Cl^--containing pipette solution (Fig. 2). With 13 mM Cl^--containing pipette solution, the reversal potential was shifted to around -60 mV (data not shown). The frequency and amplitude of sIPSCs ranged from 0.4-13.3 (4.3±0.9) Hz and 3-195 (18.1±2.0) pA, respectively (Table 1). The decay of sIPSCs was well fitted by a single exponential

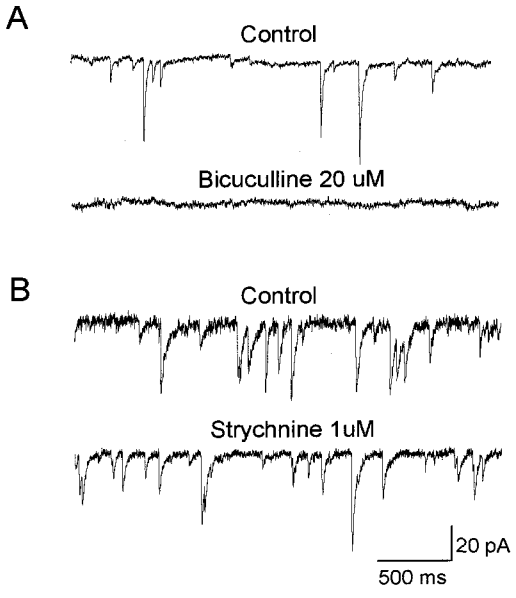

Fig. 1. sIPSCs recorded at a holding potential of −60 mV in the presence of DNQX (20 μM) and AP5 (50 μM). A) sIPSCs were completely blocked by bicuculline (20 μM) B) Application of strychnine (1 μM) had no significant effect on the frequency and amplitude of sIPSCs.

function. The decay time constant was measured in each MVN neuron for 5 to 10 sIPSCs, and the average value was 8.9±0.4 ms. The decay time constant was not correlated with the amplitude of the current, indicating that the largest currents were under adequate voltage control to assess the decay kinetics. Pharmacological isolation of the glutamatergic sEPSCs was achieved by perfusion with 20 μM bicuculline. Under these conditions, sEPSCs were generated with a multiple decay time course (Fig. 3). The slow component was blocked by AP5, while the fast component was blocked by DNQX, indicating that they were NMDA and non-NMDA mediated components, respectively. Non-NMDA mediated sEPSCs occurred at a mean frequency of 1.5±0.4 Hz and decayed with a mean of 2.8±0.2 ms. Table 1 confirms that sEPSCs and sIPSCs were easily distinguished by their amplitudes and different decay kinetics.

In the presence of bicuculline and AP5, non-NMDA-mediated sEPSCs were completely blocked by 50 μM GYKI-52466 (n=10), indicating that they were mediated primarily by AMPA-preferring receptors (Fig. 4A). Cyclothiazide (CTZ) (10 μM), a potent blocker of AMPA receptor desensitization, significantly increased the decay time constant of sEPSCs from 3.0±0.3 ms to 3.8±0.4 ms ($p<$

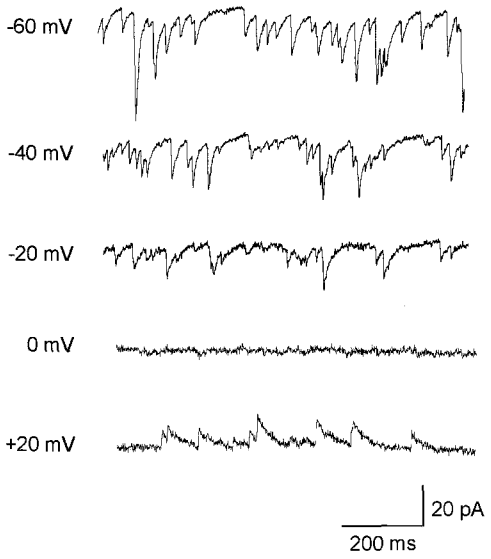

Fig. 2. sIPSCs recorded from a various holding potential. The polarity of sIPSCs was reversed at a voltage close to Cl^- equilibrium potential (0 mV). The pipette solution contained 140 mM KCl.

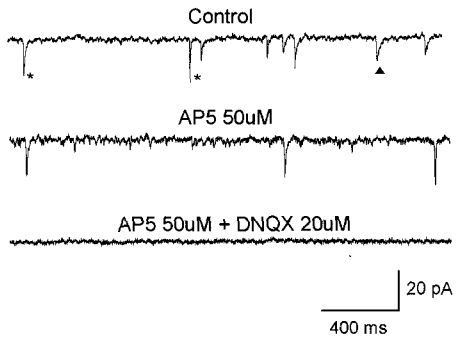

Fig. 3. sEPSCs recorded in the presence of bicuculline (20 μM). After adding 50 μM AP5, the slow components (▲) were blocked. The addition of DNQX (20 μM) blocked sEPSCs with a fast decay time (*).

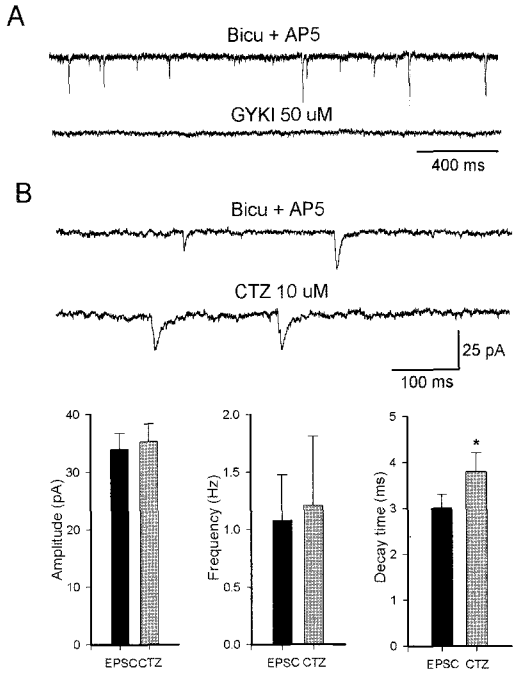

Fig. 4. Properties of non-NMDA mediated sEPSCs. A) Non-NMDA mediated sEPSCs were observed in the presence of 20 μM bicuculline and 50 μM AP5. These sEPSCs were completely blocked by 50 μM GYKI-52466 (GYKI). B) Effects of cyclothiazide (CTZ) on sEPSCs. The amplitude and frequency of sEPSCs were not changed by CTZ (10 μM), but the decay time was significantly increased.

Table 1. Comparison of sPSCs properties

	Frequency (Hz)	Amplitude (pA)	Decay time (ms)	Rise time (ms)	n
GABAergic	4.3±0.9	18.1±2.0	8.9±0.4	2.3±0.1	27/32
AMPA mediated	1.5±0.4†	13.9±1.9	2.8±0.2*	1.6±0.1*	25/29
NMDA mediated	0.8±0.3†	8.7±1.4†	9.8±0.8†	5.8±1.3††	11/30

Values are means±S.E.M. *Significant difference between GABAergic sIPSCs and AMPA or NMDA mediated sEPSCs values with $p<0.001$, †$p<0.01$. ††Significant difference between AMPA mediated and NMDA mediated sEPSCs values with $p<0.001$.

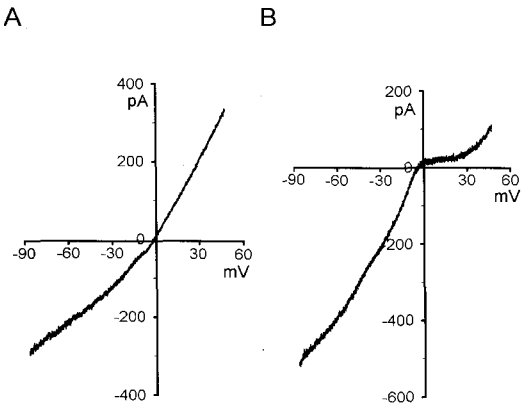

Fig. 5. Rectification properties of AMPA-induced currents in MVN neurons. A) Slightly outward rectifying I-V relationship of AMPA-induced currents. B) Inwardly rectifying I-V relationship. The control solution contained 20 μM bicuculline and 50 μM AP5. The pipette solution contained 140 mM CsCl and 5 mM QX-314. Currents were evoked by slow voltage ramps (25 mV/s) from −90 to 50 mV in the absence and presence of 50 μM AMPA. The difference current, obtained by subtracting the control current from the current evoked in the presence of AMPA, represents the AMPA-induced current.

0.05, n=18) (Fig. 4B). In contrast to the action of CTZ on the sEPSCs time course, there was no change in either the sEPSCs frequency or the amplitude, indicating that transmitter release was not significantly altered by CTZ. The I-V relationship of the sEPSCs was determined by subtracting the control currents elicited by voltage ramps from those evoked during continuous superfusion of the agonist. Fig. 5 shows the subtracted currents in response to slow voltage ramps (25 mV/s) from −90 to 50 mV. Of the 21 cells examined, 15 cells displayed a linear or slight outwardly rectifying I-V relationship after bath application of AMPA (50 μM), indicating the presence of GluR2-containing AMPA receptors (Fig. 5A). The sEPSCs frequency of these cells was 1.64±0.43 Hz. In the remaining 6 cells, the I-V relationship of the evoked current displayed inward rectification, suggesting the presence of GluR2-lacking AMPA receptors (Fig. 5B). These cells had a markedly lower rate of sEPSCs, with a mean frequency of 0.15± 0.05 Hz ($p<0.05$).

DISCUSSION

To the best of our knowledge, this is the first study to characterize spontaneous synaptic transmission and to investigate the electrophysiological evidence of AMPA-preferring receptors in the MVN. Previous studies have demonstrated that MVN neurons possess $GABA_A$, $GABA_B$, and glycinergic receptors (De Waele et al, 1995; Vidal et al, 1999). In our study, however, the sIPSCs were completely blocked by the addition of bicuculline, indicating that they are exclusively mediated by $GABA_A$ receptors and that MVN neurons do not receive a significant numbers of spontaneously active glycinergic inhibitory synapses in the early developmental stage. It is also possible that $GABA_B$ and glycine receptors are localized presynaptically. The amplitude distributions of sIPSCs were broad, ranging from 3 to 195 pA, and had several peaks, indicating strong variation in the number of vesicles released and in the postsynaptic density. Similar broad, skewed amplitude distributions have been described for a number of synapses (Bekkers et al, 1990; Lui & Tisen, 1995; Zhou & Hablitz, 1997). Unlike the sIPSCs, the sEPSC amplitudes had a narrow unimodal distribution, suggesting that only one or a few vesicles were spontaneously released at an excitatory synapse. In MVN cells from 13- to 17-day-old rats, sIPSCs and sEPSCs were characterized by significantly different amplitudes and frequencies. The sIPSCs had greater amplitudes and higher frequencies than sEPSCs, suggesting that GABAergic transmission dominates glutamatergic transmission in controlling neuronal activity of the MVN by modifying the electrotonic structure of the neurons or by directly shunting excitatory currents (Bernander et al, 1991; Salin & Prince, 1996). In addition, the decay kinetics of sIPSCs are relatively slower than those of sEPSCs, making inhibitory currents more potent during tonic synaptic excitation.

In 1 mM $[Mg^{2+}]_o$ and at a holding potential of −60 mV, only 35% of the recorded MVN neurons had NMDA mediated currents, and these were characterized by small amplitude and low frequency. This indicates that, in MVN neurons at a physiological $[Mg^{2+}]o$ and at potentials near the resting membrane potential, significantly fewer NMDA receptors contribute to the resting discharge than sIPSC and AMPA-mediated sEPSCs. In addition, our observation of a week sensitivity to $[Mg^{2+}]_o$ of NMDA receptors in MVN neurons is expected, because these neurons express the NR2C subunit which are weakly blocked by Mg^{2+}.

Fig. 4 indicates that the non-NMDA receptor component of sEPSCs is mediated primarily by AMPA-preferring receptors. The selective AMPA-preferring antagonist GYKI-52466 blocked the non-NMDA component of sEPSCs. Cyclothiazide, which potentiates responses mediated by AMPA-preferring receptors, prolonged the decay time constant of sEPSCs. Combined together, these results indicate that MVN neurons utilize mainly AMPA-preferring receptors and few, if any, KA-preferring receptors to mediate non-NMDA receptor synaptic responses. The decay of the synaptic current could be determined by the rapid removal of the transmitter, desensitization of the receptor, and the kinetics of channel relaxation (Grudt & Henderson, 1998). Although CTZ significantly prolongs the decay phase of sEPSCs, we cannot exclude the possibility that the change in sEPSC kinetics is derived from an increase in deactivation (Koike et al, 2000; Hirasawa et al, 2001).

The different glutamate receptor subunits (GluR1-4) have different physiological and pharmacological properties. For example, the I-V relationships of recombinant AMPA receptors that lack GluR2 are inward rectifying, while those that contain GluR2 have linear or slightly outward rectifying I-V relationships (Hollmann & Heinemann, 1994; Bowie & Mayer, 1995; Geiger et al, 1995; Jonas & Burnashev, 1995; Jacoby & Samuel, 2001; Liu & Cull-candy, 2002). In addition, receptors lacking GluR2 display high Ca^{2+} permeability (Bowie & Mayer, 1995; Liu & Cull-candy, 2002). In this study, more than two-thirds of the recorded neurons showed an AMPA-induced current with a linear or outward rectifying I-V relationship. This is consistent with the immunohistochemical results that show that the majority of vestibular neurons do not express GluR2 at birth, but increases its expression by up to 60~

80% during development (Pellegrini-Giampetro et al, 1992; Sans et al, 2000). Therefore, the AMPA receptors on MVN neurons would have low calcium permeability. The level of spontaneous synaptic activity can determine the subunit composition of postsynaptic receptors (Liu & Cull-candy, 2002). The expression of receptors containing GluR2 increases after high frequency stimulation (Liu & Cull-candy, 2000). If this activity-dependent switch of GluR2 subunits occurs under normal physiological conditions, one would expect differences in the rectification of synaptic currents at different synapses (Liu & Cull-candy, 2002). In this study, we found that the I-V relationship of synaptic currents in MVN neurons showed various degrees of rectification (from inwardly rectifying to linear) that were correlated with the frequency of sEPSCs. This result is consistent with the idea that intrinsic synaptic activity increases the expression of GluR2-containing AMPA receptors at synapses. The frequency of spontaneous synaptic currents in slices might have been reduced by the removal of MVN cells during slice preparation, therefore, could be lower than that in vivo. However, it is also possible that changes in synaptic activity during slice preparation could potentially alter the rectification of sEPSCs in MVN neurons.

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