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COMPARISON OF SINGLE NMDA RECEPTOR CHANNELS RECORDED ON HIPPOCAMPAL PRINCIPAL CELLS AND ORIENS/ALVEUS INTERNEURONS PROJECTING TO STRATUM LACUNOSUM-MOLECULARE (O-LM CELLS)*

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NMDA receptors participate in the glutamatergic excitation of both principal cells and GABAergic interneurons. The features of NMDA channels on specific interneurons, however, are not known. Therefore, we obtained direct measurements of single NMDA receptor channels on anatomically identified oriens/alveus interneurons projecting to stratum lacunosum-moleculare (O-LM cells) and compared them to those found on hippocampal principal cells using cell-attached recordings in *in vitro* slice preparations. The recorded channels could be blocked by ketamine, a membrane-permeable NMDA channel inhibitor. In the absence of Mg^{2+}, all O-LM cells had NMDA channels with a comparable slope conductance (~60pS) to those measured on CA1 pyramidal cells or dentate granule cells. In addition, NMDA channels with smaller conductance (43–45 pS) were also found on two O-LM cells but not on principal cells. These results suggest that at least two types of NMDA channels are expressed on O-LM cells likely reflecting distinct subunit composition.

Keywords: Cell-attached recording – pyramidal cells – granule cells – GABAergic interneuron – hippocampus

INTRODUCTION

Glutamate mediates fast excitation in cortical areas. Synaptically released glutamate activates both ionotropic and metabotropic receptors. The N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptors, has both ligand- and voltage-gated properties [17], plays crucial roles in a wide range of physiological and pathological processes including development, long-term plasticity, epilepsy, and neurodegenerative disorders [4]. These changes are largely due to the elevation of intracellular Ca^{2+} level which is triggered by the Ca^{2+} entry via NMDA channels [19]. NMDA receptor channels consist of NR1 and NR2 subunits. Four different subtypes of NR2 subunit (NR2A-NR2D) ensure the large variety in properties and several pos-

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sibilities for modulation by kinases or phosphatases such as by Ca^{2+}/calmodulin kinase type II (CaMK II) or serine threonine phosphatase 2B (calcineurin) [4, 11, 12, 21].

Cortical neuronal networks consist of two major neuron types: excitatory principal cells and inhibitory interneurons. In the hippocampal formation, the principal cells are hippocampal pyramidal cells and dentate granule cells forming about 90% of the entire neuronal population [23]. These largely uniform cells have been investigated in great detail. On the other hand, much less data are available on GABAergic interneurons forming heterogeneous neuronal populations with distinct anatomical and functional characteristics [5].

To determine the features underlying fast excitatory neurotransmission between distinct neuron types, the similarities or differences of glutamatergic receptors have to be revealed. Therefore, our aim was to compare the properties of NMDA channels playing essential roles in excitation between hippocampal principal cells and interneurons. Because of the large heterogeneity among interneurons, we have chosen an anatomically well-characterized interneuron type, the so-called O-LM cells. These GABAergic neurons have horizontally running spiny dendrites in stratum

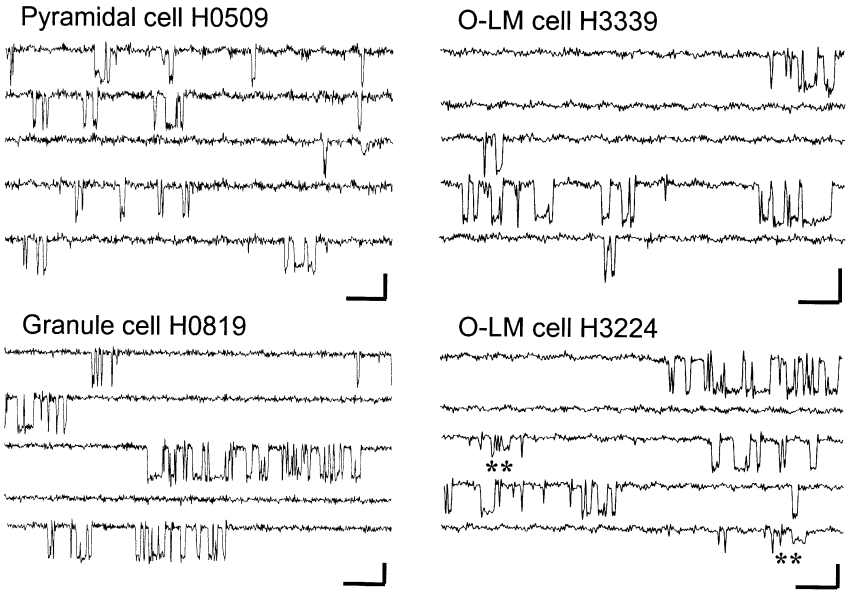

Fig. 1. Single channel recordings of NMDA receptors on hippocampal neurons. Representative traces with openings depicted as downward deflections are taken from the patches recorded on the somata of different types of neurons in the hippocampus. Channels were activated by low concentrations of specific NMDA receptor agonist L-aspartate (200–500 nM) in the presence of 10 μM glycine. In principal cells, both in pyramidal and in granule cells, only one type of channel could be recorded. In O-LM cells, similar channels could be observed, but in some cases, channels with smaller amplitude were also detected (asterisks). Vertical and horizontal bar values are 4 pA and 20 ms, respectively

oriens and alveus, while their axon arbor is restricted to stratum lacunosum-moleculare in the CA1 region of the hippocampus [7, 8, 15]. These cells are vulnerable in the kainic acid-induced model of temporal lobe epilepsy [3].

MATERIALS AND METHODS

Slice preparation

Young (20-28 d. old) male Wistar rats were decapitated under deep sodium pentobarbital anesthesia (70 mg/kg, i.p.). After opening the skull, the head was immersed into cold (~4 °C) modified artificial cerebrospinal fluid (ACSF), and the brain was removed. This ACSF contained (in mM) 126 NaCl, 2.5 KCl, 26 $NaHCO_3$, 0.5 $CaCl_2$, 10 $MgCl_2$, 1.25 NaH_2PO_4, 10 glucose and 2 mM kynurenic acid. Coronal slices (350 μm-thick) were prepared using a Lancer Series 1000 Vibratome. The slices were sagittaly bisected along the midline, and were incubated in a storage chamber in ACSF (containing 2 mM each of $CaCl_2$ and $MgCl_2$) for 30 min at 32 °C, and then the whole chamber was transferred to room temperature (22–23 °C).

Electrophysiology

Fire-polished borosilicate (KG-33, 1.5 mm o.d.; Garner Glass) glass pipettes (6–10 MΩ) were filled with the recording/extracellular solution containing (in mM): 110 Na_2SO_4, 5 Cs_2SO_4, 1.8 $CaCl_2$, 10 HEPES, 10 glucose, 1 pyruvic acid, 0.001 TTX (pH 7.2–3; final osmolarity was 290–310 mOsm). Cell-attached recordings were obtained at room temperature from hippocampal neurons using infrared DIC videomicroscopy (Zeiss Axioscope) for visualization of the target cells. Recordings were performed using an Axopatch 200B amplifier (Axon Instruments, Inc.), digitized at 88 kHz (Neurorecorder, NeuroData), and stored on videotape. Channel data were filtered at 1 or 1.5 kHz (8-pole Bessel), redigitized with a sampling rate of 10 kHz, and analyzed off-line. Details of the events detection using PAT program (courtesy of J. Dempster) with a 50% threshold-crossing algorithm are given elsewhere [13]. Data are presented as mean ±SD.

Anatomical identification of neurons

At the end of the recordings, the recording pipette was gently removed from the cell body. Using another patch pipette, which contained 135 Cs gluconate, 5 CsCl, 20 HEPES, 2 $MgCl_2$, 2 Mg-ATP and 1–1.5% biocytin at pH 7.2–3 adjusted with CsOH, we repatched the same cell and filled it up with biocytin for 5–10 min. Then slices were placed back into the storage chamber for an hour, and fixed overnight in 4% paraformaldehyde, 0.05% glutaraldehyde and 15% picric acid in 0.1 M phosphate

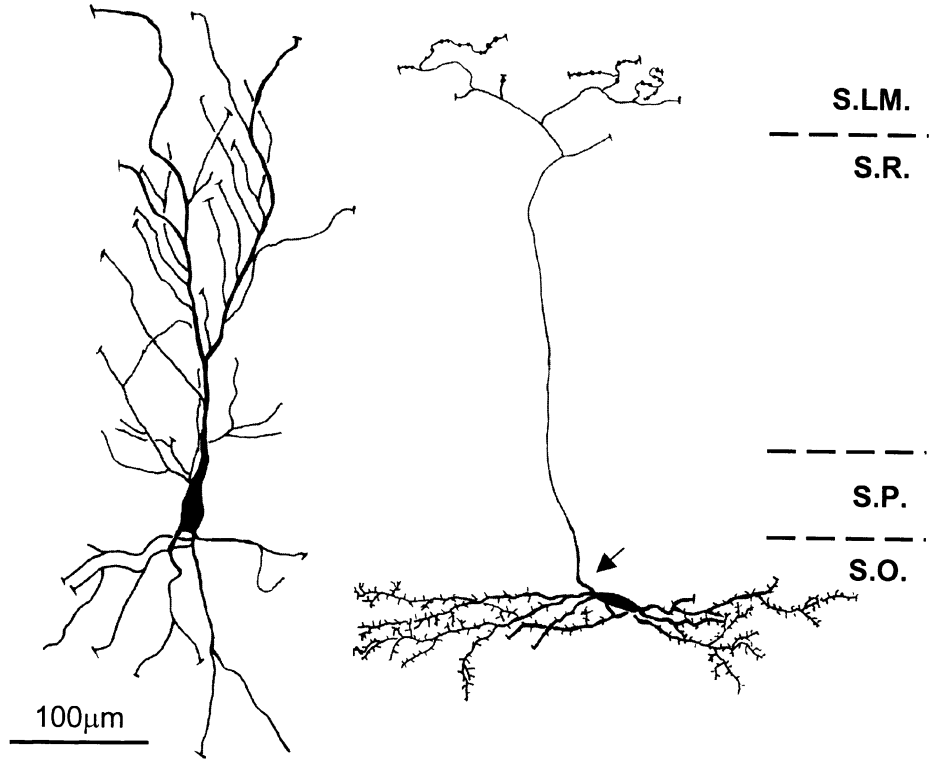

Fig. 2. Camera lucida reconstructions of a CA1 pyramidal cell and an O-LM cell. After the single channel recordings, cells were repatched with a new pipette containing biocytin and were filled for 5–10 min. The soma of the pyramidal cell (left) was located in the cell body layer, and its dendrites span all layers of the CA1 region. In contrast, the cell body and the horizontally running spiny dendrites of O-LM cell (right) were found in stratum oriens (S.O.). Its main axon originated in a proximal dendrite (arrow), and ascended toward stratum lacunosum-moleculare (S.LM.) where gave rise to axon collaterals. S.R. – stratum radiatum, S.P. – stratum pyramidale

buffer (PB, pH 7.4). The slices were resectioned at 80 μm with a Vibratome, incubated in cryoprotecting solution (0.01 M PB containing 12% glycerol and 25% sucrose) for 30 min, freeze-thawed three times above liquid nitrogen, and treated with 0.5% H_2O_2 in 0.1 M PB for 30 min to reduce endogenous peroxidase activity. Injected neurons were visualized using avidin-biotinylated horseradish peroxidase complex reaction (ABC) with nickel-intensified 3,3'-diaminobenzidine as chromogen (dark blue reaction product). After dehydration and embedding in Durcupan, the neurons were identified based on their dendritic and axonal arbors.

RESULTS

In Mg^{2+} free solution, we investigated the NMDA type glutamate receptors on the somata of both hippocampal principal cells, i.e. pyramidal cells of the CA1 region and granule cells of the dentate gyrus, and of O-LM cells (oriens/alveus interneurons projecting to stratum lacunosum-moleculare; Fig. 2). Single NMDA receptor channels were activated by low concentrations of a specific agonist, L-aspartate (200–500 nM) in the presence of 10 μM glycine to prevent the pronounced desensitization in cell-attached patches [6, 10]. The macroscopic appearance of NMDA channels was similar on O-LM cells compared to those recorded on the principal cells (Fig. 1). On two O-LM cells, however, a single channel type with small conductance (asterisks on Fig. 1) was also observed besides those channels having large conductance. This kind of channel was not seen on hippocampal principal cells (n = 12).

To identify the single channels activated by L-apsartate in our patch recordings, 100–150 μM ketamine was added to the bath solution to selectively block NMDA

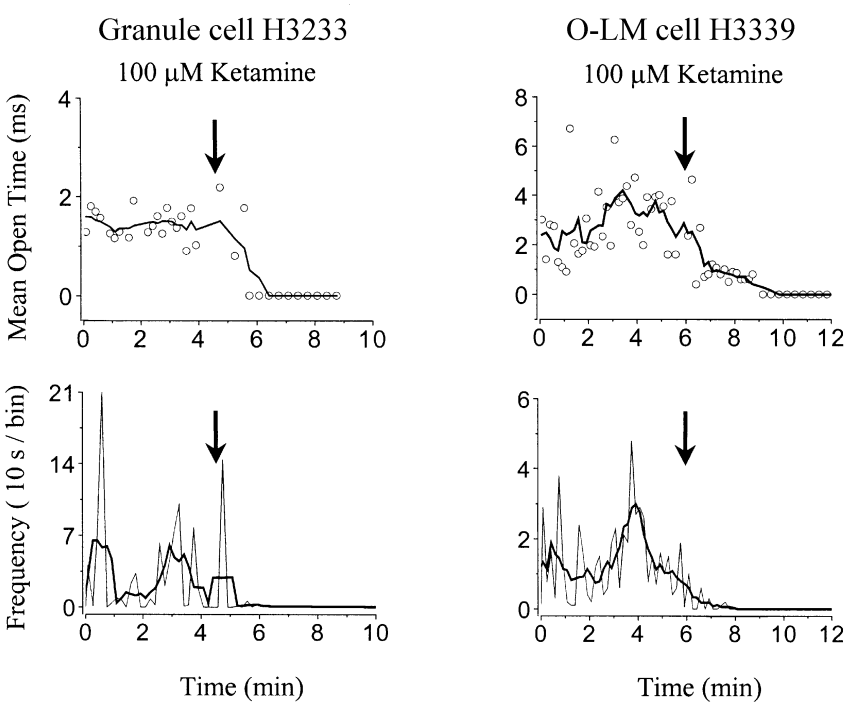

Fig. 3. Identification of NMDA receptor channels on hippocampal neurons. Within 2–6 min following bath application of a membrane permeable NMDA channel blocker ketamine (100–150 μM), all channel activity in the patches recorded on a granule cell and an O-LM cell was completely blocked. After the measurement, cells were filled with 1% biocytin using a new electrode and *post hoc* anatomically identified by ABC reaction (see methods)

channels [18]. After 2–6 min of bath application of ketamine, single channel openings in the patches were completely diminished on all tested neurons (n = 5, Fig. 3) proving that the L-aspartate activated channels are indeed NMDA receptors.

Next we compared the slope conductance of NMDA channels on hippocampal principal neurons and on O-LM cells. In the absence of Mg^{2+}, we measured the amplitude of single NMDA channels at different holding potentials. The slope conductance of the channels was determined by fitting a linear regression to the points of the current-voltage (I–V) plot. The slope conductance was 60 ± 1.7 pS for principal cells (n = 5) and 58 ± 1.1 pS for O-LM cells (n = 4) (Fig. 4). In addition to NMDA channels with large conductance on O-LM cells, the conductance of single NMDA channels with smaller amplitude (43 pS and 45 pS, respectively) was also determined (Figs 1, 4).

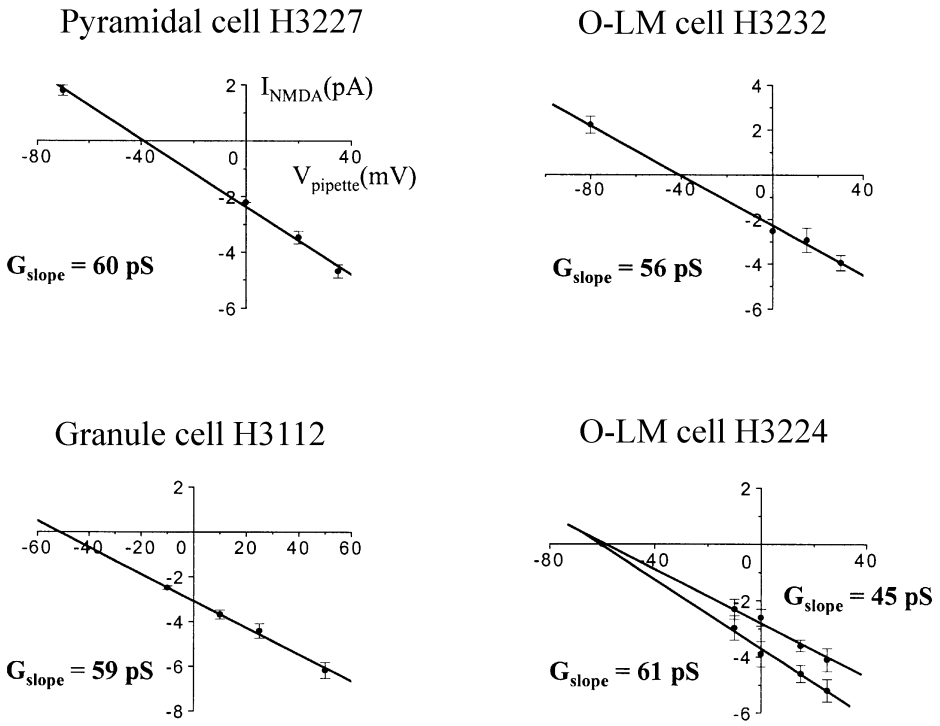

Fig. 4. The slope conductance of NMDA channels was comparable in hippocampal principal cells and O-LM cells. In Mg^{2+} free solution, the current-voltage relationship of NMDA channels was linear in all tested neurons. Representative examples of both principal cells and O-LM cells are shown. Note that the reversal potential of single NMDA channel currents is variable among cells which might reflect their different tolerance for the external solution (see methods). In an O-LM cell (H3224), an NMDA channel with smaller slope conductance (see Fig. 1) was also identified in addition to the NMDA channels observed in hippocampal principal neurons

DISCUSSION

The slope conductance of large amplitude NMDA channels was found to be comparable between principal neurons and O-LM cells, which suggests a similar or perhaps the same subunit composition. The conductance of NMDA receptor channels is determined by the type of NR2 subunit incorporated in addition to the essential NR1. Results obtained in expression systems have shown that the presence of NR2A or NR2B subunit in a channel gave rise to a large conductance (40–50 pS). In contrast, channels containing NR2C or NR2D subunits had small conductance (30–40 pS) [4]. After age P20, CA1 pyramidal cells express only NR2A and NR2B in addition to NR1 subunits [9, 16]. Interneurons, however, may express NR2D subunit besides those found in pyramidal cells [1, 22]. Taken together, these data suggest that NMDA channels with large conductance recorded on principal cells (60 ± 1.7 pS) or O-LM cells (58 ± 1.1 pS) in our study likely contain NR2A or NR2B subunits, while NMDA channels with smaller conductance (45 and 43 pS) found on O-LM cells suggest the existence of NR2D subunit as well [22].

The NMDA channel conductances obtained in our cell-attached recordings in slice preparation (~ 60 pS) is consistently larger than the values of ~ 50 pS established in dissociated cell preparation [6, 10] or in outside-out patch recordings [2]. This discrepancy is due, at least in part, to the deficiency of NMDA channels to be connected with actin filaments in outside-out patches and perhaps in dissociated cells as well [2].

Phosphorylation and dephosphorylation of NMDA channels by CaMK II and calcineurin play a pivotal role in the induction of long lasting changes in synaptic efficacy such as long-term potentiation (LTP) or depression (LTD). These enzymes are present in hippocampal principal cells, and control the activity of single NMDA channels [13, 20]. In contrast, GABAergic cells do not express either CaMK II or calcineurin, and consequently NMDA receptors are not regulated by them as it has been directly shown by Sik et al. [20]. The only form of synaptic plasticity recorded from O-LM cells to date is LTD, which has been shown to be of presynaptic origin [14]. These data emphasize that expression of NMDA channels by itself is not sufficient for the induction of postsynaptic LTP or LTD, but the use-dependent regulation of these channels is critical for cellular function.

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