Variable Kainate Receptor Distributions of Oriens Interneurons

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Submitted 19 December 2005; accepted in final form 7 June 2006

Yang, Ellen J., Alexander Z. Harris, and Diana L. Pettit. Variable kainate receptor distributions of oriens interneurons. J Neurophysiol 96: 1683–1689, 2006. First published June 14, 2006; doi:10.1152/jn.01332.2005. Interneuron kainate receptor (KAR) activation regulates normal network activity and modulates cell excitability. As a result, determining the subcellular distribution of KARs in a cell-specific manner is a necessary step toward understanding their role in network function. We have functionally mapped synaptic and extrasynaptic dendritic KARs on hippocampal oriens interneurons using local photolysis of caged glutamate. We find that the majority of trilaminar and oriens lacunosum-moleculare (O-LM) oriens interneurons synapse on CA1 pyramidal dendrites within lacunosum moleculare and stratum radiatum where they modulate excitatory inputs and action potential propagation (Larkum et al. 2001; Sandler and Ross 1999; Tsubokawa and Ross 1997; Vetter et al. 2001).

METHODS

Electrophysiology

Standard techniques were used to prepare 300-μm-thick slices from the hippocampus of 2- to 3-wk-old rats. Whole cell voltage-clamp recordings (~70 mV) were made from CA1 SO interneurons. The patch pipette was filled with a cesium gluconate solution containing (in mM) 100 gluconic acid, 5 EGTA, 5 MgCl₂, 2 ATP, 0.3 GTP, and 40 HEPES; pH to 7.2 with CsOH. Slices were superfused at room temperature with oxygenated physiological saline (in mM: 119 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.5 CaCl₂, 1 NaH₄PO₄, 26.2 NaHCO₃, and 11 glucose). Recordings were accepted only if the holding current was <100 pA. Data were collected and analyzed with Igor Pro (WaveMetrics, Lake Oswego, OR).

Photolysis experiments

The multiline UV output of a continuous emission 5-W krypton ion laser (Coherent, Innova 302) was delivered, by optical fiber, through an Olympus × 40 water-immersion objective to form a 5.7-μm uncaging spot. (Pettit et al. 1997; Wang and Augustine 1995). Each uncaging location was sampled three times before moving to a new location.

Density measurements

Surface area (A = 2πrh) was calculated by assuming the dendrite was a cylinder with length equal to the size of the uncaging spot (5.7 μm) and a diameter measured as the profile of fluorescence intensity across the width of the process (Pettit and Augustine 2000). Cells with dendrites that did not produce a clear intensity profile were not analyzed. The somal uncaging area was calculated using previously measured beam characteristics (Wang and Augustine 1995) to estimate the upper and lower surface area of the soma exposed to photolysis (498 μm²).

Histology

Cells were filled for 1 h with neurobiotin (1%). After completion of a mapping experiment. Slices were postfixed in 4% paraformaldehyde, stained for neurobiotin with avidin-biotin-horseradish peroxidase (HRP) (Vector Laboratories, Burlingame, CA), developed with 3,3′-diaminobenzidine tetrahydrochloride, and sectioned on the cryostat (100 μm). A × 25 oil-immersion lens was used for camera lucida drawings. Of the 61 interneurons mapped, 43 cells had neurobiotin fills sufficient to allow identification of the axon terminations. Three types of interneurons were found: O-LM (n = 17), trilaminar (n = 17), and bistratified (n = 9).

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KAR distribution

Regional variations in dendritic KAR current density were functionally mapped using local photolysis of bath applied 4-methoxy-7-nitroindolinyl (MNI)-caged L-glutamate (200 μM; Tocris, Ellisville, MO). Whole cell recordings were obtained from SO interneurons in the presence of voltage-gated sodium and calcium channel blockers (TTX, 1 μM; cadmium, 50 μM; cadmium, 50 μM). TABLE 1. Of the 61 cells studied, 43 had axon fills sufficient to allow for classification of interneuron subtype.

<table>
<thead>
<tr>
<th>Interneuron Subtype</th>
<th>Continuous</th>
<th>Focal</th>
<th>Asymmetric</th>
<th>Total</th>
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<tr>
<td>O-LM</td>
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<td>5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Trilaminar</td>
<td>11</td>
<td>6</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Bistratified</td>
<td>9</td>
<td></td>
<td>9</td>
<td>17</td>
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FIG. 1. Most oriens lacunosum-moleculare (O-LM) and trilaminar interneurons have a uniform kainate receptor (KAR) current density. A: photolytic currents elicited by repeatedly moving from site A to B. Traces are consecutive 3 trials at each location. Overlaid traces are averages from each trial. B: live confocal image and glutamate responses from a multipolar horizontal stratum oriens (SO) interneuron after GYKI application (50 μM). Circles indicate position and diameter of the UV light spot. Traces are an average of 3 responses at each location. C: plot of current density for the cell in B. D: normalized current density plot for all cells (n = 23). E: KA/AMPAR current ratio for all cells (n = 20). F: camera lucida drawing of an SO interneuron whose axon termination indicates that it is an O-LM cell (SP, stratum pyramidale).
FIG. 2. KAR-mediated currents are restricted to localized hot spots in a subpopulations of O-LM and trilaminar interneurons. A: SO interneuron with AMPA currents (white; average of 3 trials) but no KAR currents after GYKI application (50 μM; red). B: another cell where subsequent application of 5 μM NBQX eliminated all remaining current (green). C: another cell with responses only at branch points and soma. D: mean current density and KA/AMPAR ratio for responding hot spots (n = 13 cells). E: camera lucida drawing of a trilaminar cell exhibiting this response profile (SP, s. pyramidale).
μM). Cesium was included in the pipette solution to improve voltage clamp by blocking potassium channels. Dendrites were visualized by including Oregon-green BAPTA (200 μM) in the recording pipettes, and maps were created by sequentially positioning the UV light spot (~6 μm) at nonoverlapping locations (>12 μm apart).

Photolysis (1–2 ms; 5–10 mW) evoked inward synaptically and extrasynaptic receptor currents in the presence of selective N-methyl-d-aspartate (NMDA; d-APV, 50 μM) and GABA (picrotoxin, 50 μM) receptor blockers. Uncaging locations were identified on the live image by measuring their distance along the dendrite and natural anatomical landmarks. This approach produced reliable current responses at each location (Fig. 1A). After creating a mixed AMPA/KAR map, KARs were isolated by bath application of the AMPA receptor antagonist GYKI 53655 (GYKI, 50 μM), and KAR maps were produced by revisiting each location. Subsequent bath application of the nonselective AMPA/kainate receptor antagonist NBQX (5 μM) blocked all photolytic current, confirming that the currents were caused by KAR activation (n = 7). Live images of the cell were acquired with a confocal microscope (Olympus Fluoview 300). We examined three different SO interneuron subtypes: O-LM, trilaminar, and bistratified. Comparison of multiple dendritic maps revealed three distinctive KAR distributions.

**Diffuse distribution interneurons**

These interneurons are characterized by KAR-mediated responses at all locations on the dendrites and cell body, with horizontal multi- or bipolar dendrites confined to the SO (Fig. 1B; n = 28). Application of GYKI reduced mixed AMPA/KAR current amplitude [143 ± 8 (SE) pA; n = 147 spots] at an individual location by 75.4 ± 3.8% (n = 10 cells) leaving small amplitude KAR currents (35 ± 5 pA; n = 147 spots). Current density was calculated by dividing current amplitude by the surface area at each location (Fig. 1C). To allow comparisons between cells and for variance in glutamate concentration caused by differences in uncaging time or laser power, values were normalized to the dendritic density in the most proximal bin (<50 μm; Fig. 1D; n = 23). Figure 1, C and D, shows that KAR current density increased with distance along the dendrite, doubling in density at the most distal locations. This increase in density was mirrored by the KA/AMPA ratio, which also increased with distance along the dendrite (Fig. 1E). Both O-LM (n = 12) and trilaminar (n = 11) interneurons expressed this diffuse distribution of KAR current density (Fig. 1F; Table 1).

**Focal density interneurons**

Another group of interneurons are characterized by robust AMPAR-mediated currents and little or no response to caged agonist following GYKI application (50 μM). These cells also have horizontal multipolar dendrites in the SO. This group contains two types of cells. One type has AMPA currents and no KAR currents (Fig. 2A; n = 7), whereas the other group is characterized by small KAR currents restricted to “hot spots” at discrete locations such as the soma and branch points (Fig. 2, B and C; n = 13). Application of NBQX (5 μM) blocked the remaining KAR-mediated current (Fig. 2B). Average KAR current densities and KA/AMPA ratios at “hot spots” were similar to that seen on the diffuse distribution interneurons (Fig. 2D; n = 13). Morphological analysis showed that nonresponders and focal responders were either O-LM (n = 5) or trilaminar cells (n = 6; Fig. 2D; Table 1) (McBain et al. 1994; Sik et al. 1995).

**Asymmetric density neurons**

These cells have vertical multipolar dendrites extending into SO and s. radiatum. They exhibit KAR currents on all dendrites located within SO, but the dendrites extending into s. radiatum do not have photolytically elicited KAR currents (Fig. 3, A–D; n = 13). S. radiatum dendrites did respond to glutamate because they expressed AMPA currents. Increasing laser power over these dendrites also failed to yield currents (Fig. 3C; n = 4). Similar attempts to increase the sensitivity of our assay such as increasing uncaging time or caged glutamate concentration (600 μM) also failed to elicit KAR currents. Density plots and KAR/AMPA current ratios along the length of all active dendrites look similar to the uniform density plots. KAR densities and ratios increased with distance along the dendrite (Fig. 3, D and E; n = 9). As with uniform density neurons, GYKI application reduced peak current amplitude 78 ± 2.3% (n = 9). Of the three cells examined, only bistratified interneurons (Sik et al. 1995), which synapse in the s. radiatum (Halasy et al. 1996), showed this distribution (Fig. 3F; n = 9).

**Discussion**

Interneurons constitute a diverse group of cells that set the level of excitation within the brain. Tremendous effort has gone into classifying characteristic features of interneuron subtypes to better understand their physiological roles. Here we have used photolysis of caged glutamate to functionally map KAR currents in the dendrites of CA1 hippocampal oriens interneurons. We found both interneuron subtype-dependent and independent KAR distributions. The asymmetric KAR distribution is restricted to the bistratified subtype of interneurons. However, we also found that some interneuron subtypes share KAR distributions and can have more than one distribution. O-LM and trilaminar cells, which, respectively, affect theta and gamma rhythms, can have an absence of KAR currents, diffuse, or focal KAR current distributions. This suggests that the distribution of KAR-mediated currents may...
constitute an additional dimension of subspecialization of the interneurons in the hippocampus.

We have previously shown that synaptic and extrasynaptic AMPA/KARs on CA1 interneurons exhibit current densities that increase with distance from the cell body, despite synaptic innervation densities that seem homogenous (Pettit and Augustine 2000). This gradient may act to amplifying distal synaptic signals, compensating for distance-dependent signal attenuation (Eder et al. 2003; Magee and Cook 2000; Pettit and Augustine 2000). Although the increase was modest, we found a similar gradient of KAR current density in SO interneurons. Given that we measured currents at the soma, it is possible that dendritic currents generated at distal dendritic sites were attenuated, causing us to underestimate KAR current density. However, the sizable length constant (1.1) of these cells (Lacaille and Williams 1990), and the fact that our measurements were made from dendritic regions within 300 μm of the soma, suggest that distance-dependent attenuation of current amplitude should not exceed 25%.

Focal density interneurons were characterized by an absence of KAR-mediated currents over much of the dendritic tree. Although we see some cells that have no KAR currents, we cannot definitively state they are different from the focal response cells, because it is not possible to sample every region of dendrite on a single cell. As a result, we placed both response profiles in the same group. The lack of KAR currents is surprising because we have seen currents on all other cell types tested including CA1 and CA3 pyramidal cell dendrites where density should be low because they do not have synaptic KARs (Castillo et al. 1997; Cossart et al. 2002; Lerma et al. 1997). We were initially concerned that our technique was not sensitive enough to detect KARs at low densities. As a result, we tried tripling laser power, uncaging time, or glutamate concentration. These manipulations failed to elicit KAR currents. While focal density cells may represent a separate population of O-LM and trilaminar neurons, there were no anatomical differences between these cells and diffuse distribution cells. Selective positioning of KARs at “hot spots” such as branch points may be a way to achieve maximal impact for a small number of receptors. Branch points are locations where backpropagating action potentials fail and subthreshold currents may be attenuated (Larkum et al. 2001; Vetter et al. 2001). Therefore depolarizing KARs at this location could act to boost signals (Sandler and Ross 1999).

Bistratified interneurons (Freund and Buzsaki 1996) have uniform current densities on all dendrites except those descending into s. radium. This lack of current in the s. radium cannot be caused by signal attenuation because these cells are characterized by failure of KAR current within 20 μm of the soma. Conversely, uniform distribution dendrites produce substantial currents at distances >275 μm (Figs. 2 and 3). Given that dendrites enter the s. radium over shorter distances than 275 μm, this cannot explain the lack of currents on radium dendrites. While it is not clear why the KAR density is so low on s. radium dendrites, these cells may provide a unique opportunity for studying receptor targeting mechanisms. One consequence of excluding KARs from the s. radium is a potential reduction of synaptic strength. Because these currents have a slower decay (τKAR = 52.8 ± 3.9 ms; τAMPAR = 15.9 ± 1.1 ms; n = 20), inclusion of KARs in the synapse should substantially increase charge transfer and synaptic efficacy. As a result, excitatory inputs to s. radium dendrites may be less heavily weighted than inputs to SO dendrites. Interestingly, s. radium dendrites are the only SO cell dendrites likely to receive input from CA3 (Buhl et al. 1996), and exclusion of KARs may limit the impact of CA3/Schaffer collateral synapses.

ACKNOWLEDGMENTS

We thank Drs. Reed Carroll and Lori McMahon for helpful comments on the manuscript.

GRANTS

This work was supported by National Institutes of Health Grants 1RO1 NS-44399, HD-01799, and DK-07513 and the Whitehall Foundation.

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