Freshly Isolated Hippocampal CA1 Astrocytes Comprise Two Populations Differing in Glutamate Transporter and AMPA Receptor Expression

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We have shown previously that process-bearing GFAP+ astrocytes freshly isolated from rat hippocampus CA1 and CA3 regions are heterogeneous in ion channel expression and K⁺ uptake capabilities, such that two distinct populations of astrocytes can be described (Zhou and Kimelberg, 2000). In the present study, we report that glutamate transporter (GT) currents can only be measured from one type of these freshly isolated hippocampal CA1 astrocytes [variably rectifying astrocytes (VRAs)] but were not detectable in the second type of astrocyte [outwardly rectifying astrocytes (ORAs)] showed an AMPA receptor current density (ORAs) showed an AMPA receptor current density (65 pA/pF) that was 42-fold higher than VRAs (1.3 pA/pF). In contrast, the GABA_A currents were of comparable current density in both types. The specificity of these differences makes it unlikely that they are attributable to preparative damage. Therefore, these findings strongly indicate that, within a single region of the hippocampus, GFAP+ astrocytes comprise a functionally diverse population that are qualitatively different in their functional glutamate transporter and quantitatively different in their functional AMPA receptor expression. This heterogeneity implies that GFAP+ astrocytes may participate in or modulate glutamate synaptic transmission differently.

Key words: fresh cell isolation; hippocampus; GFAP+ astrocytes; glutamate transporter; AMPA receptor; patch-clamp

Accumulating evidence suggests that astrocytes may play both supportive and active roles in brain. This is particularly being shown in relation to excitatory synaptic transmission. Astrocytic glutamate transporters (GTs) have been shown to be primarily involved in maintaining low extracellular glutamate (Glu) levels in vivo (Rothstein et al., 1996; Anderson and Swanson, 2000). Astrocytes also have AMPA receptors (AMPA-Rs) originally shown in astrocytes in primary culture (Bowman and Kimelberg, 1984; Kettenmann et al., 1984) and then in situ and in freshly isolated preparations (Steinhäuser and Gallo, 1996). AMPA-R has been demonstrated to be potentially functional in situ studies because perisynaptic glutamate concentrations may transiently rise to a sufficient level to activate astrocytic AMPA-Rs (Dzubay and Jahr, 1999). Astrocytes have been shown to respond to glutamate released from synaptic terminals in the hippocampal CA1 region by increases in intracellular Ca²⁺ via activation of both metabotropic and ionotropic glutamate receptors (Porter and McCarthy, 1996). One of the many possible consequences of astrocytic AMPA-R activation has been suggested to be the release of n-serine from astrocytes, which can then potentiate the postsynaptic NMDA receptor glycine site as an endogenous ligand to modulate synaptic transmission and plasticity (Baranano et al., 2001).

Electrophysiological studies of astrocytes identified morphologically in situ (D’Ambrosio et al., 1998) and in GFAP+ cells in freshly isolated preparations (Zhou and Kimelberg, 2000) have indicated that hippocampal astrocytes are heterogeneous in their ion channel expression. The question we asked here is whether these differences in ion channel expression are also associated with differences in glutamate transporter and AMPA-R currents.

We studied glutamate transporter and AMPA-R currents using freshly isolated astrocytes (FIAs) and a fast perfusion drug delivery system to resolve the Glu-induced current under conditions favorable to activation of both astrocytic AMPA-R and glutamate transporter currents. As a comparison, we also analyzed GABA_A receptor-mediated current because GABA_A has been shown in acutely isolated astrocytes also from hippocampus (Fraser et al., 1995). Our data show that GFAP+ astrocytes freshly isolated from the hippocampal CA1 region of postnatal day 7 (P7) to P35 rats comprise two subpopulations with quite different functional properties in regard to functional glutamate transporter currents and AMPA-Rs, in addition to the previously identified difference in regard to ion channel currents.

MATERIALS AND METHODS

Cell isolation. The procedure for preparation of FIAs has been described in detail previously (Zhou and Kimelberg, 2000) and is performed in accordance with a protocol approved by the Albany Medical College Institutional Animal Care and Use Committee. Briefly, hippocampal slices were prepared from 7- to 35-d-old Sprague Dawley rats and maintained in a Ca²⁺-free solution containing (in mM): 124 NaCl, 5 KCl, 2 MgSO₄, 1 Na-pyruvate, 26 NaHCO₃, and 10 glucose (bubbled with 95% O₂–5%CO₂ for 1 hr). Subsequently, the slices (six to eight from each animal) were transferred into standard artificial CSF solution containing 24 U/ml papain and 0.24 mg/ml cysteine and bubbled with 95% O₂–5%CO₂ at 22°C for 30 min. The enzymatic digestion was halted by thorough washing of the slices in Ca²⁺-free solution. The slices were then stored in the Ca²⁺-free solution for at least 1 hr before trituration.
Morphology and current profiles of ORAs and VRAs. A. Imaging of a freshly isolated astrocyte during recording with Lucifer yellow dye (0.3%), in the pipette, showing bushy processes extending from the cell body. The processes are not very distinct because there is some folding back of the processes and because of the out-of-focus fluorescence halo, as this photograph was taken through the nonconfocal Nikon Diaphot microscope we used in the recording set up. B, E, Membrane currents induced by voltage steps (50 msec) from −160 to +60 mV (20 mV increments) with a NO3−-based pipette solution (see Materials and Methods). ORAs (B) are characterized by a dominant expression of outward Ik and Ina plus small inward INa− currents (see inset below). VRAs (E) are characterized by a symmetric expression of inward and outward potassium currents. C, F, When K+ channel-mediated currents were completely masked by the substitution of pipette KCl with CsCl, an ORA (C) and a VRA (F) were identified based on their identical bushy morphology but marked different membrane capacitances (10.5 pF in recording C and 38 pF in F). The INa− is shown in higher resolution in C. INa− currents were never observed in VRAs (F). D and G are recordings C and F, respectively, at higher resolution and after off-line compensation for leak and capacitance.

In some preparations, FIAs were prepared without the 1 hr preenzymatic and postenzymatic incubation in Ca2+-free solution to verify that these preparation steps do not cause the differences seen in FIAs. Before recording from the freshly isolated astrocytes, the CA1 stratum radiatum was dissected out under the microscope to avoid the inclusion of any part of the dentate gyrus, which may contain GFAP+ stem cells (Liu et al. 2000). The dissected stratum radiatum of the CA1 region was then triturated into the recording chamber. Only “bushy,” process-bearing astrocytes were selected for recording, which in our previous studies were shown to be both GFAP mRNA+ and GFAP+ (Zhou and Kimelberg, 2000; Schools and Kimelberg, 2001).

Solutions and drugs. The standard bath solution contained (in mM): 150 NaCl, 5 KCl, 2 MgCl2, 2 CaCl2, 10 glucose, and 10 HEPES, pH adjusted to 7.4 with NaOH. The pipette solutions contained (in mM): 140 KNO3 (or KSCN), 0.5 CaCl2, 1 MgCl2, 5 EGTA, 10 HEPES, 3 Mg-ATP, and 0.3 Na-GTP, pH adjusted to 7.25 with KOH. To measure GABA receptor-mediated Ca2+ currents without contamination by K+ channel-mediated currents, the bath solution contained (in mM): 119 NaCl, 5 BaCl2, 2 CaCl2, 3 mM CsCl, 5 KCl, 2 MgCl2, 10 glucose, and 10 HEPES. Also 140 mM KCl was substituted by an equimolar amount of CsCl in the pipette solution.

Electrophysiology. Whole-cell membrane currents were measured by an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) and sampled by a TL-1 DMA Interface (Axon Instruments). Data acquisition was by pClamp 6.0.4 software (Axon Instruments). Low-resistance patch pipettes (3–6 MΩ) were fabricated from borosilicate capillaries (outer diameter, 1.5 mm; Warner Instruments, Hamden, CT) using a Flaming/Brown Micropipette Puller (model P-87; Sutter Instruments, Novato, CA). Capacitance and series resistance (Rs) compensation (40–50%) were used to improve voltage-clamp control. Membrane capacitance (Cs) and series resistance (Rs) were determined by a depolarizing test pulse from −70 to −60 mV (10 msec, filter at 10 kHz, sampling at 30 kHz). In some experiments, 0.3% Lucifer yellow was added to the pipette solution to more distinctly show the complete astrocyte morphology. Drugs were rapidly applied to the established whole-cell patches through square tubes of the Perfusion Fast-Step System (SF-77; Warner Instruments) controlled by programmed data acquisition protocols. Experiments were performed at room temperature (−20–24°C). The microscope used was a Nikon (Tokyo, Japan) Diaphot inverted stage equipped with phase contrast and fluorescence optics.

Data analysis. Data are given as means ± SE. The dose–response curve was fitted by the following equation: \( I = I_{\text{max}} \left[ \frac{1}{1 + \left( \frac{[E_d]}{[E]_0}\right)^n} \right] \). Significance differences were evaluated according to the Student’s t test. The level of significance was set at \( p < 0.05 \).

RESULTS

Morphology and ion channel expression of freshly isolated astrocytes

Outward rectifying astrocytes (ORAs) and variably rectifying astrocytes (VRAs) could not be morphologically distinguished; both were bushy, process-bearing cells resembling the classical descriptions of protoplasmic astrocytes (Privat and Rataboul, 1986). A representative bushy astrocyte filled with Lucifer yellow dye during the recording is shown in Figure 1.A. These cells are ≥95% GFAP+ by immunocytochemistry (Schools and Kimelberg, 2001). In Figure 1, B and E, we show the two distinct ion channel profiles that we reported previously (Zhou and Kimelberg, 2000). One type, the ORA, is characterized by a dominant expression of voltage-gated outward potassium currents (Ik and Ik,p) and inward sodium current (INa−) (Fig. 1B, inset). The second type, the VRA, is distinguished by a pronounced expression of leak K+ current (IK,leak) and low-density expression of Ik,p plus IK,dr (Zhou and Kimelberg, 2000). To further verify that
$I_{\text{Na}}^+$ is a characteristic feature of ORA and also to rule out the possibility that VRA type astrocytes also express $I_{\text{Na}}^+$ but this small $I_{\text{Na}}^+$ is obscured by the abundant $I_{\text{K}_{\text{brane}}}$ we performed experiments in which $K^+$ was substituted by $Cs^+$ in the pipette solution (see Materials and Methods) to mask the activation of any $K^+$ channel-mediated current (Fig. 1C,F). Under these conditions, VRAs show a larger membrane capacitance (32.8 ± 5.4 pF; $n = 10$) than ORAs (10.2 ± 2.7 pF; $n = 12$), as we described previously (Zhou and Kimelberg, 2000). However, although enhanced $I_{\text{Na}}^+$ could be seen in ORAs ($n = 12$) (Fig. 1D), no $I_{\text{Na}}^+$ could be detected in VRAs ($n = 10$) (Fig. 1G). This result confirmed our previous observations that $I_{\text{Na}}^+$ seems a diagnostic criteria in distinguishing ORAs from VRAs.

**Selecte expression of glutamate transporter currents by VRAs**

The translocation of Glu via GTs is driven by the transmembrane Na$^+$, K$^+$ gradients, which generates a small but measurable coupled charge movement. It also has been shown that GT activation is associated with an anion conductance that is ~10-fold larger than the GT-coupled charge movement (Fairman et al., 1995; Bergles and Jahr, 1997). In the dominant astrocytic GT isomorph, GLUT-1 (also termed EAAT2), the uncoupled anion conductance displays a similar kinetics to coupled charge movement and thus likely precisely reflects Glu transport (Otis and Kavanaugh, 2000). We used the highly permeant anions NO$_3^-$ or SCN$^-$ in the pipette solution to predominantly measure the anion conductance (Fairman et al., 1995; Wadiche et al., 1995). Under these recording conditions, the basic ion channel profiles for both ORA and VRA did not differ from KCl-based solution. However, the whole-cell $I-V$ relationship of VRAs showed a 10.8 ± 7.8 mV positive shift in reversal potential ($n = 25$; data not shown) compared with the KCl-based pipette solution, suggesting the existence of a persistent resting inward conductance when NO$_3^-$ is the major anion in the pipette solution. As demonstrated in previous studies, this resting conductance is an indicator of anions leaving the cell via the GT-associated anion pathway (Bergles and Jahr, 1997; Levy et al., 1998; Otis and Jahr, 1998; Otis and Kavanaugh, 2000).

During fast application of a saturating concentration of L-Glu (10 mM) at −70 mV holding potential, ORAs showed a typical astrocytic AMPA-R response (Seifert et al., 1997), characterized by a fast activation and a rapid and almost complete desensitization (Fig. 2A, dashed trace). The peak amplitude was −498 ± 50.6 pA ($n = 21$). VRAs showed a smaller peak current amplitude of −101 ± 25 pA but a larger steady-state current amplitude of −22 ± 6.9 pA ($n = 11$) (Fig. 2B, dashed trace). The Glu-evoked current in ORAs was completely abolished by the selective AMPA-R antagonists NBOX (10 µM) plus GYKI$_{52466}$ (25 µM) (Fig. 2A). For VRAs, however, the peak current amplitude was only partially blocked by these antagonists (reduced by 50.6 ± 10.7%; $n = 11$). Additionally, the steady-state currents were enhanced by an average of 40 ± 12% at the end of Glu pulse (result from one cell is shown in Fig. 2B, solid trace). This NBOX–GYKI$_{52466}$-insensitive current had an initial peak current of −54 ± 6 pA and a steady-state current of −43.7 ± 4.1 pA ($n = 11$), which are very similar to the kinetics of GT currents identified from patches excised from CA1 astrocytes from hippocampal slices (Bergles and Jahr, 1997).

We further determined that the NBOX–GYKI$_{52466}$-resistant currents seen in VRAs were GT-associated currents by the following experiments. First, substitution of extracellular Na$^+$ by Li$^+$ completely abolished this current ($n = 3$) (Fig. 3A). Second, THA, a transportable, competitive inhibitor of all of the GTs so far identified, induced a sustained inward current and also prevented Glu from inducing any additional current at the −70 mV holding potential ($n = 4$) (Fig. 3B). We did not see any THA-induced currents in ORAs ($n = 5$; data not shown). Finally, assuming the NBOX–GYKI$_{15466}$-insensitive current is purely carried by NO$_3^-$ and the relative permeability of $P_{\text{Cs}}/P_{\text{NO}_3^-} = 0.07$ (Wadiche and Kavanaugh, 1998), the reversal potential of this current should approach the $E_{\text{NO}_3^-}$ of ~70 mV. As shown in Figure 3, C and D, the Glu-induced NBOX–GYKI$_{52466}$-resistant
absolutely blocked by NBQX− from P25 also include a gain of GTs, so we tested this by recording from channel expression (Brockhaus and Deitmer, 2000). This could fl

(A1)–

inward currents (after off-line subtraction of inward and outward potassium currents) persisted up to approximately +70 mV (n = 3). These data together support activation of Na+−-dependent GT currents on VRAs.

Absence of GT currents in ORAs is independent of the anions used or age of animals

Because GT currents were not detected in ORAs under our standard condition, we next asked whether ORAs express functional GT isoforms but at a relatively low density. Thus, their GT currents might only be seen with anions having a higher permeability than NO3−. Therefore, we also used SCN−, which gives the largest anion conductance for all of the identified GTs so far (Palacin et al., 1998). As shown in Figure 4, A1 and A2, the Glu-induced current with SCN− in the pipette was also completely blocked by NBOX−–GYKI52466 in ORAs (n = 3).

We observed that, in older animals (P18–P30), 27% of ORAs additionally express a small inward K+ currents (IKin) (see Figure 4B1) (Zhou and Kimelberg, 2000). Such an ion channel profile has been suggested recently, reflecting a developmental switch in ion channel expression (Brockhaus and Deitmer, 2000). This could also include a gain of GTs, so we tested this by recording from ORAs from P25–P35 rats that show IKin. Glu at 1 mM induced an average peak current of −477 ± 172 pA (n = 8) and a steady-state current of −9 ± 6.1 pA (n = 8), closely matching the peak and steady-state current values of ORAs measured from the earlier developmental stages (see previous section of Results). This Glu-induced current (Fig. 4B2) was also completely blocked by NBOX−–GYKI52466 (Fig. 4B3), showing that no GT currents are present in the ORAs isolated from older animals that show significant IKin (Fig. 4B1). These data further supports the view that ORAs are a persistent and distinct astrocytic subtype.

Although the specificity we observed from FIAs seems unlikely to be attributable to preparative damage because ORAs and VRAs experienced the same isolation procedure, we did reduce the time of the isolation procedure. We found both the specificity of ion channel profiles (n = 2 for ORAs; n = 4 for VRAs) and GT and AMPA-R responses remained (data not shown) with the omission of the 2 hr incubation of slices in Ca2+−-free solution (see Materials and Methods).

GTs in VRAs show varied sensitivity to DHK and show high-affinity glutamate uptake

To explore whether different GT isoforms contribute to the GT current in VRAs, we tested the DHK sensitivity in VRAs isolated from P7–P14 rats. We found that the specific GLT-1 inhibitor DHK at 300 µM induced a sustained resting outward current in the VRAs (13.4 ± 1.2 pA; n = 7), presumably attributable to blockade of a resting anion conductance associated with GTs, as seen in excised astrocytic soma patches (Bergles and Jahr, 1997). Figure 5 is a representative recording showing the upward shift in basal current when 300 µM DHK was applied before Glu and then the inhibition of both the peak and steady-state GT currents during a Glu pulse. DHK at 300 µM inhibited the peak and steady-state GT currents by 41 ± 9.3% (range of 12–82%; n = 7) and 36.4 ± 10.6% (range of 0–72%; n = 7), respectively. The varied partial inhibition of GT currents by DHK is consistent with a coexistence of GLT-1 and the other glial type GT isoform GLAST, as seen at this developmental stage (Furuta et al., 1997; Lehre and Danbolt, 1998). P7–P14 is the time period that GLT starts to replace GLAST as the dominant GT isoform (Furuta et al., 1997).

The peak to steady-state current amplitude ratios in our whole-cell recordings were ~1.3, which is lower than the value recorded from small excised patches, typically ~4 (Bergles and Jahr, 1997). Because the FIAs have an intricate morphology, a synchronized peak response of all of the GTs currents in one cell may be practically impossible, even with Glu applied to the isolated cells using a rapid delivery system. Thus, to estimate the Glu EC50,
only the steady-state GT currents were used for our dose–response analysis. In representative recordings shown in Figure 6A, the steady-state current amplitude values of the GT currents of VRAs were measured at different Glu concentrations and normalized to the current obtained for 1 mM Glu from the same cell to give the dose–response curve shown in Figure 6B. The best fit to the Hill equation gave a Glu $EC_{50}$ of $4 \pm 1.1 \mu$M with a Hill coefficient ($n_H$) of 0.6, supporting a high-affinity uptake of Glu by VRA GTs. This $EC_{50}$ is approximately threefold lower than the value analyzed from steady-state GT currents of excised patch of somatic membrane of hippocampal astrocytes ($EC_{50}$ of $13 \mu$M) but with a comparable $n_H$ of 0.59 at the same developmental stage (Bergles and Jahr, 1997). Possible reasons for the difference are covered in Discussion.

ORAs show a higher AMPA-R current density than VRAs

We noticed in measuring the GT currents (Fig. 2) that the currents sensitive to AMPA antagonists were much smaller in VRAs than in ORAs for cells from P7–P14 rats. These differences are summarized in Table 1, which includes data from P7–P14 and P25–P30 rats. It can be seen that the average peak AMPA-R current density of ORAs for both age groups is 42-fold greater than VRAs.

ORAs and VRAs show comparable GABA$_A$ receptor currents

The opposite differences in GTs and AMPA-R current expression, together with our finding of distinct ion channel profiles, argues against the differences in ORAs and VRAs are attributable to physical damage, which seems unlikely to produce such two distinct populations of cells. To further explore whether, or to what extent, the enzyme treatment can affect the measurement of receptors, transporter, or ion channel proteins, we measured GABA-mediated currents because GABA$_A$ receptors show the highest sensitivity to papain treatment in hippocampal neurons in situ (McCarren and Alger, 1987). In these experiments, we used a Cs$^+$-based pipette solution to block K$^+$ channel-mediated currents. Therefore, ORAs and VRAs were identified based on the criteria of the presence of $IN_{A,-}$ in ORAs (Fig. 1C) and the absence of $IN_{A,+}$ in VRAs (Fig. 1F). As shown in Figure 7, $AI$ and $BI$, 1 mM GABA induced a robust inward current in both ORAs ($-829 \pm 259$ pA; $n = 6$) and VRAs ($-1092 \pm 359$ pA; $n = 5$), with similar activation and desensitization kinetics. This GABA-induced current was inhibited similarly by the selective GABA$_A$ antagonist bicuculline (100 $\mu$M) (Fig. 7A,2B; Table 2). Also, the selective GABA$_A$ agonist muscimol (50 $\mu$M) induced similar inward currents in both ORAs and VRAs (Fig. 7A,3B; Table 2). This expression of GABA$_A$ current in both ORAs and VRAs is in agreement with the study by Fraser et al. (1995) using freshly isolated hippocampal GFAP+ astrocytes. Our overall analysis revealed that the GABA$_A$ receptor current density is 3.3-fold higher in ORAs as compared with VRAs (Table 2), but this difference was not statistically different. The overall pharmacology profiles were the same (Table 2). These results indicate that papain treatment did not differentially alter the characteristics of functional GABA$_A$ receptors in ORAs and VRAs, if they are similarly expressed in both types.
DISCUSSION

FIAs show heterogeneity in functional glutamate transporter expression

A large body of evidences suggests that the majority of presynaptically released Glu is taken up by astrocytes (Anderson and Swanson, 2000). However, it has not been shown previously that a group of protoplasmic astrocytes within a specific brain region, which are also GFAP immunoreactive, have no functional GTs. Because the GT currents were not detectable in ORAs from older animals, the lack of functional GTs appears not to be developmentally restricted but represents a characteristic feature of ORAs. This heterogeneity requires a reconsideration of the diversity of astrocytes in terms of their function in glutamate homeostasis.

We select cells in our preparation based on a bushy, process-bearing morphology, and these cells are always GFAP+ immunocytochemically (Zhou and Kimelberg, 2000; Zhou et al., 2000). Heterogeneity for GT current expression has not been reported

Table 1. ORAs show abundant AMPA-R current densities compared with VRAs

<table>
<thead>
<tr>
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<th>AMPA-R currents (pA)</th>
<th>AMPA-R current density (pA/pF)</th>
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<tr>
<td>ORA (P7–P14)</td>
<td>−498 ± 51 (n = 21)</td>
<td>55.0 ± 5.6 (n = 21)</td>
</tr>
<tr>
<td>ORA (P25–P35)</td>
<td>−477 ± 172 (n = 8)</td>
<td>54.5 ± 3.2 (n = 8)</td>
</tr>
<tr>
<td>VRA (P7–P35)</td>
<td>−47 ± 17* (n = 11)</td>
<td>1.3 ± 0.3* (n = 11)</td>
</tr>
<tr>
<td>ORA/VRA</td>
<td>10.5</td>
<td>42</td>
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*A Indicates significant differences between ORA and VRA (Student’s t test; *p < 0.05). For ORA/VRA calculation; ORAs from both P7–P14 and P25–P35 were included.

Figure 7. GABA<sub>A</sub> receptor currents are comparable in ORAs and VRAs. A1–A3 and B1–B3 are recordings from an ORA and a VRA, respectively, at holding potential of −70 mV. In A1 and B1, a 0.5 sec 1 mM GABA pulse induced robust inward currents in both cells with a similar desensitization time course. At the end of the GABA pulse, the initial peak current (A<sub>P</sub>) desensitized 47% in A1 and 40% in B1. A2 and B2 show the similar inhibition of GABA-activated currents by the selective GABA<sub>A</sub> antagonist bicuculline (10 μM). The GABA-activated currents were reduced by 52 and 64% in an ORA (A2) and a VRA (B2), respectively. A3 and B3 were the responses induced by the selective GABA<sub>A</sub> receptor agonist muscimol (50 μM). In A3 and B3, the muscimol-activated peak current amplitudes amounted to 61 and 64% of the GABA induced currents. All recordings in A and B represent the same respective cells.

Table 2. GABA<sub>A</sub> currents are comparable in ORAs and VRAs

<table>
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<tr>
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<th>ORA</th>
<th>VRA</th>
<th>ORA/VRA</th>
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<tr>
<td>GABA (1 mM)</td>
<td></td>
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<tr>
<td>( A_P ) (pA)</td>
<td>−829 ± 259 (n = 6)</td>
<td>−1092 ± 359 (n = 5)</td>
<td>0.76</td>
</tr>
<tr>
<td>CD (pA/pF)</td>
<td>107.8 ± 19.7 (n = 6)</td>
<td>32.4 ± 2.2 (n = 5)</td>
<td>3.3</td>
</tr>
<tr>
<td>( A_P/CD )</td>
<td>56.5 ± 4.8</td>
<td>69.3 ± 10</td>
<td></td>
</tr>
<tr>
<td>Muscimol (50 μM)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>( A_P ) (pA)</td>
<td>−719 ± 222 (n = 6)</td>
<td>−840 ± 442 (n = 5)</td>
<td>0.86</td>
</tr>
<tr>
<td>CD (pA/pF)</td>
<td>98.0 ± 33.4 (n = 6)</td>
<td>21.6 ± 7.0 (n = 5)</td>
<td>4.5</td>
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<td>( % ) of inhibition of GABA (1 mM)-induced current</td>
<td>58 ± 10 (n = 6)</td>
<td>69 ± 8 (n = 5)</td>
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Values are in mean ± SEM with cell numbers in parentheses. \( A_P \) and \( A_A \) are peak and current amplitudes at the end of GABA pulses, respectively. CD, Current density calculated for capacitance differences. None of the apparent differences between ORAs and VRAs were significant by Student’s t test.
from comparable studies on hippocampal astrocytes in situ (Bergles and Jahr, 1997, 1998; Lüschet al., 1998; Diamond and Jahr, 2000). Possible reasons might be that these investigators identified astrocytes based on the criteria of the cell showing a low input resistance, very negative membrane potential, and dominant $K_{\text{OHM}}$. VRAs from FIAs show all of these properties (Zhou and Kimmelberg, 2000). Therefore, VRAs may well represent the astrocytes selected in the in situ studies just mentioned. However, bushy, process-bearing protoplasmic astrocytes in slices exhibiting similar properties to our ORAs, i.e., dominant voltage-gated K$^+$ and Na$^+$ conductances, and relatively low resting membrane potentials have also been described (Bordey and Sontheimer, 1997; McKhan et al., 1997; D’Ambrosio et al., 1998). Thus, there are no reasons to doubt their existence in situ.

The ion current profiles and high AMPA-R current densities of ORAs are similar to cells in vivo that are positive for the chondroitin sulfate proteoglycan NG2 (Bergles et al., 2000) and are usually referred to as oligodendrocyte precursor cells (Ong and Levine, 1999). We therefore double stained our preparation for both NG2 and GFAP and found that the ORAs and VRAs selected on the basis of their morphology as bushy astrocytes all stained for GFAP but never for NG2. This was found in three preparations from P8–P24 animals (G. P. Schools, M. Zhou, and H. K. Kimelberg, unpublished observations). A large number of cells in the preparation did stain for NG2, but their morphology was quite different and resembled the population of GFAP− "complex" cells described previously in a publication from our laboratory that also had current profiles resembling those of ORAs (Zhou et al., 2000). These cells in our preparation have a small round soma and short fine processes that were not as extensive as those reported for NG2+ cells in slices (Bergles et al., 2000). In our current preparations, some of these NG2-positive cells often had long fine processes. It appears that considerable lengths of these fine processes may be removed during isolation and/or staining, perhaps because they are more delicate than the thicker processes present on the GFAP+ astrocytes.

An immunocytochemical study at the electron microscopic (EM) level reported that GTs varied in their density within and between astrocytes but that no astrocytes were unlabeled (Chaudhry et al., 1995). The absence of functional GT currents in ORAs is inconsistent with this observation. However, in the EM study, the antibody raised against an amino acid fragment of GT showed that GT currents are present in GFAP+ astrocytes. Therefore, the in situ finding that VRAs are selected in the in situ studies just mentioned. However, bushy, process-bearing protoplasmic astrocytes in slices exhibiting similar properties to our ORAs, i.e., dominant voltage-gated K$^+$ and Na$^+$ conductances, and relatively low resting membrane potentials have also been described (Bordey and Sontheimer, 1997; McKhan et al., 1997; D’Ambrosio et al., 1998). Thus, there are no reasons to doubt their existence in situ.

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selectively activate NMDA receptor in adjacent synapses (Kullmann and Asztely, 1998). Morphological studies show that ~40% of hippocampal synapses are not surrounded by astrocytic processes (Ventura and Harris, 1999), a striking difference between this region and the cerebellum in which the dendritic spines of Purkinje cells are completely covered by astroglial sheaths (Spacek, 1985). Also, if some of the 60% of hippocampal synapses surrounded by astrocytic processes include processes from ORAs, these synapses could maintain a higher local Glu concentration for constant postsynaptic NMDA activation.

The fact that numerous synapses are not invested by astrocytic processes (Ventura and Harris, 1999) and that ~50% of GFAP+ astrocytes in CA1, in P7–P35 animals, consist of ORAs (Zhou and Kimelberg, 2000), may also explain why the hippocampus is one of the brain regions highly vulnerable to glutamate release under pathological conditions (Stoltenburg-Diderg, 1994).

In conclusion, we have shown that freshly isolated GFAP+ astrocytes from the CA1 region of the hippocampus show a remarkable heterogeneity in their expression of functional GT current, which correlates inversely with expression of AMPA-R currents. In contrast, GABA_A receptor currents are comparable in both types of astrocytes. Together with our previous findings of marked differences in K+ and Na+ channel expression (Zhou and Kimelberg, 2000), this implies a marked division of labor between morphologically identical astrocytes in these important characteristics. Hypotheses regarding the functional significance of these two types of cells will, at a minimum, require knowledge of how they are arranged relative to other cell types in the CA1 region. These questions need to be resolved by histological techniques using suitable markers for ORAs and VRAs.

**REFERENCES**


