Analysis of AMPA Receptor Properties During Postnatal Development of Mouse Hippocampal Astrocytes

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Seifert, Gerald, Min Zhou, and Christian Steinhäuser. Analysis of AMPA receptor properties during postnatal development of mouse hippocampal astrocytes. J. Neurophysiol. 78: 2916–2923, 1997. Glial cells in the mammalian brain express various types of voltage- and ligand-gated ion channels, including glutamate receptors (GluRs) of the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-subtype. In the present study we followed developmental changes in the functional properties of AMPA receptor (AMPA-R) channels expressed by astrocytes of the mouse hippocampus between postnatal days (P) 5–35 to learn more about the physiological significance of these glial receptors. A fast concentration clamp technique was applied to cells acutely isolated from the CA1 stratum radiatum subregion to quantitatively analyze rapidly activating and desensitizing receptor responses. The equilibrium responses of glutamate and kainate differed between P5 and P12. Although the maximum current induced by kainate was almost the same at all developmental stages, a steep rise in the maximum glutamate response was observed within the same time range. Between P5 and P12 there was an increase in the potentiation of AMPA-R currents with cyclothiazide (CTZ); at the same time, the dissociation kinetics of CTZ became significantly slower. These changes in the pharmacological properties suggested a variation in splice variant expression. With proceeding maturation, we observed an increase in the degree of desensitization of the glutamate- and AMPA-induced receptor currents. In addition to the shift in flip/flop splicing, these findings could hint at a developmental regulation of RNA editing in the arginine/glycine site. Altogether, the present results demonstrate changes in astrocystic AMPA-R functioning early in postnatal development, although after P12 the receptor properties remained almost constant. Although the overall Ca²⁺ permeability did not vary during development, the prolonged receptor opening in the early postnatal period causes an enhanced Na⁺/Ca²⁺ influx into the immature astrocytes. This could influence glial proliferation and differentiation during CNS ontogenesis.

INTRODUCTION

Glutamate is the major excitatory neurotransmitter in the CNS that activates a large variety of ionotropic and metabotropic receptors (for review, see Hollmann and Heinemann 1994). In addition to neurons, glial cells were also shown to express ionotropic glutamate receptors (GluRs) (see reviews by Kimelberg 1988; Steinhäuser and Gallo 1996). The close association of glial and neuronal synaptic elements suggests that glial GluRs are activated under physiological conditions. Recently, it was shown that astrocytes in the hippocampus express GluR of the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-subtype that are Ca²⁺ permeable (Jabs et al. 1994; Porter and McCarthy 1995; Seifert and Steinhäuser 1995). A subsequent reverse transcription/polymerase chain reaction (RT/PCR) analysis of AMPA receptor (AMPA-R) mRNA expressed in immature astrocytes revealed a preferential expression of the subunits GluR2/GluR4 (Seifert et al. 1997). Besides its classical role in neurotransmission, the action of glutamate is important for the regulation of neuronal circuitries and cytoarchitecture during neuronal differentiation (for review, see McDonald and Johnston 1990). The AMPA-R mediated action of glutamate has distinct effects on morphological rearrangements in neurons (Mattson et al. 1988) and astrocytes (Cornell-Bell et al. 1990). Furthermore, recent findings indicate that AMPA-Rs play a role in the regulation of the proliferation of glial cells (Gallo et al. 1996; Liu and Almazan 1995; LoTurco et al. 1995).

Four genes encode the AMPA-prefering subunits GluR1–4 (Hollmann et al. 1989; Keinänen et al. 1990). The relative abundance of the GluR2 subunit determines the Ca²⁺ permeability of the AMPA-R channels in native cells (Geiger et al. 1995; Jonas et al. 1994). A further functional complexity is produced by the process of alternative splicing (Sommer et al. 1990). These splice variants, called flip and flop, control the receptor desensitization (Mosbacher et al. 1994) and thus modulate the kinetics of synaptic transmission as well as the vulnerability of neurons to excitotoxins. Both regulatory mechanisms of AMPA-R expression probably play a crucial role in brain development.

Initially, ligand binding studies demonstrated developmental changes in the expression of AMPA and kainate receptors in various brain areas (Insel et al. 1990; Miller et al. 1990). In the hippocampus the number of binding sites showed a consistent increase within the first 4 wk of postnatal development. Subsequent in situ hybridization (Monyer et al. 1991; Pellegrini-Giampietro et al. 1992; Standley et al. 1995) and Northern analysis (Durand and Zukin 1993) confirmed that expression of AMPA-R subunits is altered during ontogenesis. Although these reports mainly focused on receptor expression in neurons, much less information is available on glial cells. Thus in the present work we followed the functional changes of AMPA-Rs in astrocytes of the hippocampus during early postnatal development. By using electrophysiological methods, we found differences in pharmacological and kinetic properties of the receptor channels between P5 and P35.

METHODS

Cells were acutely isolated as previously described (Steinhäuser et al. 1994b). Female mice were anaesthetized (50% O₂–50% CO₂) and decapitated, their brains were dissected out, washed, and the
hemispheres were cut into slices in frontal orientation with a vibratome. Slice preparation was performed at 6°C in nominally Ca²⁺-free external solution. Subsequently, the hippocampi were removed from the slices and then were incubated in oxygenated papain (24 U/ml, supplemented with 0.24 mg/ml cysteine) containing bath solution (22°C). The incubation times were 15, 30, and 45 min at postnatal day (P)5, P12, and P35, respectively. After washing, the hippocampi were stored in oxygenated Ca²⁺-free external solution at 6°C. The stratum radiatum of the CA1 region was dissected out and the cells were disaggregated under microscopic control in standard bath solution by using fire polished Pasteur pipettes. Only cells without indications of osmotic swelling were selected for the experiments.

Solutions, electrodes, and drugs

The standard bath solution contained (in mM) 150 NaCl, 5 KCl, 2 MgSO₄, 2 CaCl₂, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 10 glucose. In Ca²⁺-free solutions, CaCl₂ was omitted and 1 mM sodium pyruvate was added. In 50 mM Ca²⁺ solutions, NaCl and MgSO₄ were replaced by N-methyl-d-glucamine (NMG) to ensure a constant osmolarity. The pH of the external solutions was adjusted to 7.4. The pipette solution contained (in mM) 90 KCl, 40 KF, 10 HEPES, and 3 ATP. For the analysis of I-V relations of the agonist activated currents, KCl and KF were replaced by 90 mM CsCl, 30 mM CsF, and 10 mM tetraethylammonium chloride (TEA). The liquid junction potential measured for these solutions was between 10 and 15 mV and remained constant throughout the experiment (deviation was <3 mV after breaking the seal at the end of the experiment). All data were corrected to account for this offset. The pH of the pipette solutions was 7.25.

AMPA (hydrobromide salt) and cyclothiazide (CTZ) were purchased from RBI (Natick, MA) and all other salts and reagents from Sigma (St. Louis, MO). CTZ was dissolved in dimethyl sulfoxide at 20 mM before dilution in the bath solution. In all CTZ experiments, the corresponding control solutions contained 0.5% dimethyl sulfoxide. Recording pipettes were produced from borosilicate capillaries (Hilgenberg, Malsfeld, Germany).

Recording setup and fast application technique

Membrane currents were measured with the patch-clamp technique in the whole cell mode. Current signals were amplified (EPC-7 amplifier, List Electronics, Darmstadt, Germany), filtered at 3 or 10 kHz, sampled at 6 or 30 kHz, and monitored with TIDA software (HEKA, Lambrecht, Germany), running on a 486 computer. Low-resistance patch pipettes (3–4 MΩ) were used for the patch-clamp investigations. The input resistance in the cells under study ranged between 0.4 and 4 GΩ (n = 65); the differences between P5, P12, and P35 were not statistically significant. Before capacity cancellation, membrane capacitance (Cm) and series resistance (Rs) were determined from the current transients evoked by a 10-mV test pulse depolarizing the cells from −70 to −60 mV (sampling rate 30 kHz, filter 10 kHz). The time constants were determined by fitting a single exponential to the current decay. The mean values of Cm were 7.5 ± 4.5 (SD) pF (n = 59, P5), 6.8 ± 3.6 pF (n = 37, P12), and 4.4 ± 2 pF (n = 50, P35; Fig. 2A, Table 1). Cm and Rs compensation (40–50%) were used to improve voltage clamp control. Because the receptor currents recorded were typically <500 pA, residual voltage errors resulting from incomplete compensation did not exceed 3 nV. The measurements were performed at room temperature. Agonists were applied by using a fast concentration clamp technique (Seifert and Steinhäuser 1995), which enabled a complete solution exchange within 2–3 ms. The 20–80% exchange time was 0.9–1.2 ms. However, because the desensitization of the glutamate-induced cur-

![FIG. 1. Current pattern and immunocytochemical identification of immature astrocytes acutely isolated from CA1 stratum radiatum. A: typical current pattern of immature astrocytes obtained at different developmental stages as indicated. Cells were stepped for 50 ms to increasing depolarizing potentials, ranging from −160 to 20 mV with 10-mV increments. Holding potential was −70 mV. B: after recording, the cells were immunolabeled for S100β to confirm their astrocytic origin. Note that S100β-positive and -negative cells possessed a similar pattern of voltage-activated currents.](http://jn.physiology.org/)

rents was very rapid, it is possible that the corresponding glutamate peak amplitudes have been underestimated.

Cell identification

Isolated glial cells were identified according to their electrophysiological and immunocytochemical properties. Cells were grouped as follows: P5 (P3–5), P12 (P9–12), and P35 (P30–35). They showed a characteristic pattern of voltage-gated currents (Fig. 1A) (Steinhäuser et al. 1992, 1994b). As a matter of routine, all cells were checked in the current clamp mode to confirm the absence of action potentials (not shown; cf. Seifert and Steinhäuser 1995; Seifert et al. 1997). In contrast to the findings in situ (Kressin et al. 1995) however, no consistent up-regulation of inwardly rectifying K⁺ currents (Iₖᵢ) was observed in cells isolated later in development. This discrepancy to the in situ data could be the result of a proteolysis-mediated block of Iₖᵢ, as was recently observed for hyperpolarization-activated currents in neocortical neurons (Budde et al. 1994). Alternatively, it could indicate that Iₖᵢ channels were mainly located at distal glial processes (Wilson and Chiu 1990) that were lost during cell isolation.

Previous immunocytochemical analysis suggested that these cells had an astroglial origin (Akopian et al. 1996; Kressin et al. 1995). To substantiate this assumption, we tested the cells for their immunoreactivity to S100β, a calcium binding protein expressed primarily by gray matter astrocytes that is considered an astroglial marker both in the developing and mature nervous system (Barger et al. 1992). After electrophysiological characterization, the cell
maximal effect (EC50), the agonist concentration at half-maximal response curves were fitted by the equation

\[ I(t) = I_\text{ss} + A \exp(-t/\tau_1) + B \exp(-t/\tau_2) \]

where \( I_\text{ss} \) is the steady-state current at \( t = \infty \), \( A \) and \( B \) are amplitude factors, and \( \tau_{1,2} \) are the corresponding time constants. Dose-response curves were fit by the equation

\[ I = I_\text{max} \left\{ \frac{1}{[1 + (\text{EC}_{50}/[\text{kainate}])^n]} \right\} \]

where \( I_\text{max} \) is the response at saturating concentration; one-half maximal effect (EC50), the agonist concentration at half-maximal receptor activation; \( n \), the Hill coefficient; and [kainate], the kainate concentration. The degree of receptor desensitization was defined by

\[ \text{Desensitization} = 100\% \left\{ \frac{I_\text{peak} - I_\text{ss}}{I_\text{peak}} \right\} \]

where \( I_\text{peak} \) is the maximal receptor current and \( I_\text{ss} \) is the steady-state current in the presence of the agonist, which was determined 300 ms after the onset of application.

All data are given as means ± SD. Significance differences were evaluated according to the Student’s t-test. The level of significance was set at \( P = 0.05 \).

Results
Developmental changes in current density and agonist potency

At a membrane potential of −70 mV, fast application of glutamate and AMPA activated rapidly desensitizing inward currents in hippocampal glial cells, whereas the kainate responses were almost nondesensitizing (insets in Fig. 2B). We used saturating agonist concentrations to elicit maximal receptor responses.

To compare the receptor current densities at different developmental stages, the peak currents were divided by the corresponding \( C_m \) for each individual cell. With increasing age, \( C_m \) decreased (Table 1; Fig. 2A), probably reflecting an increasing loss in Arborization because of the enhanced enzymatic/mechanical stress necessary to suspend the cells from the more aged tissue (Oh et al. 1995). The maximal glutamate response per cell surface area significantly increased between P5 and P12, while remaining constant with further development. In contrast, no such age-dependent differences were observed in the maximal kainate current densities (Table 1; Fig. 2B).

To get the dose-response relationship for kainate, the agonist concentration was varied logarithmically, and the resulting currents were normalized to the respective peak current. A single or a double exponential function was fitted to the data (Fig. 2B). The agonist concentrations for which the response was 50% of the peak response \( (\text{EC}_{50}) \) was calculated from each curve. The data analysis allowed us to determine for each individual cell, the corresponding steady-state currents (1 mM kainate) were calculated from decay of test pulses was set at 100 ms.

Table 1: Developmental changes in membrane capacitance and agonist equilibrium responses

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<td>( C_m ) (pF)</td>
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<td>Glutamate current density (1 mM, pA/pF)</td>
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<td>104.6 (21)</td>
<td>86.9 (15)</td>
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<td>Kainate current density (1 mM, pA/pF)</td>
<td>14.2 (14)</td>
<td>17.4 (12)</td>
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Values are means ± SD with cell numbers in parentheses. \( C_m \), membrane capacitance; P, postnatal days. * Significance of differences.

Table 2: Developmental changes in membrane capacitance and agonist equilibrium responses

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nyst was rapidly applied at $-70$ mV. This protocol evoked non-desensitizing inward currents at all concentrations tested (10–5,000 $\mu$M). The currents were normalized to the responses elicited by 5 mM kainate. Between P5 and P35, the kainate $E_{50}$ increased from 260 $\mu$M at P5 to 470 $\mu$M at P35 (Fig. 3), whereas at P12, an $E_{50}$ of 350 $\mu$M was found (Seifert and SteinhaÈ user 1995). Comparing the dose-response curves at P5 and P35 revealed significant differences in the concentration range between 250 $\mu$M and 1 mM, indicating a decrease in kainate potency with maturation. The corresponding hill coefficients were 1.2 (P5) and 1.1 (P35).

**Age-dependent modulation of AMPA receptors by cyclothiazide**

Previous work on recombinant receptors has shown that the amplitudes of glutamate responses were influenced much more by alternative splicing than kainate currents. The relative affinity of the agonists, however, was not different for the flip and flop receptor forms (Sommer et al. 1990). Thus the age-dependent differences obtained in the present experiments could indicate changes in splice variant expression. To test this hypothesis, we used CTZ, a substance that differentially modulates the flip and flop forms of AMPA-Rs (Partin et al. 1993), with the flip forms being stronger potentiated than the flop forms (Partin et al. 1994, 1995). After a control application of 1 mM kainate, the cells were preincubated in CTZ-containing solution and were again exposed to the agonist. In all cases, we observed an increase in the kainate currents by CTZ as compared with the control application. However the potentiation was clearly dependent on the stage of postnatal development (Fig. 4A). At P5, the currents increased only by 395 ± 106% (SD; $n = 10$) and significant higher values were obtained in glial cells at P12 (637 ± 208%, $n = 13$) and P35 (629 ± 181%, $n = 18$) (Fig. 4B).

Besides its distinct modulatory effect at the AMPA-R splice variants, the affinities of CTZ to the flip and flop forms differ significantly (Partin et al. 1995). The slow dissociation of CTZ from the flip forms causes a marked delay of the recovery from CTZ modulation (Fleck et al. 1996; Partin et al. 1994). We analyzed the recovery of glutamate responses from CTZ potentiation to get further information on developmental changes in alternative splicing of AMPA-R in the hippocampal astrocytes (Fig. 5). Therefore, after CTZ application, the cells were exposed to glutamate for 5 min. During the very first seconds after CTZ removal, the glutamate responses were almost stationary, with the desensitization after 1 s reaching <10% (Fig. 5B, insets; Table 2). The subsequent current decay could be well described by a double exponential. Table 2 gives the fast ($\tau_{fast}$) and slow ($\tau_{slow}$) time constants together with their relative amplitudes for the three age groups. The portion of fast and slow dissociation kinetics was ~50% throughout the time range investigated. At P5 however, $\tau_{slow}$ was significantly faster than in the older cells, resulting in a more complete recovery from potentiation 300 s after CTZ removal (97.2 ± 2.3%). No dissociation time constants in the ms range were observed in any of the astrocytes analyzed.

**Receptor current kinetics**

Both the changes in the amplitudes of equilibrium responses of kainate versus glutamate and the increase in CTZ potentiation of the kainate responses beyond P5 were compatible with the assumption of a switch in flip/flop expression in the maturing astrocytes. The gating properties of the AMPA-R channels also critically depend on subunit composition and alternative splicing (Geiger et al. 1995; Mosbacher et al. 1994; Partin et al. 1994). The desensitization time constants of AMPA- and glutamate-induced currents were ~7–9 ms and did not vary between P5 and P35 (Table 3; Fig. 6). Despite a pronounced heterogeneity at P5, we observed a significant increase in the degree of desensitization of the glial AMPA and glutamate currents in the older cells (Table 3; Fig. 6B). The AMPA results make it very unlikely that our results were obscured by glutamate transport mechanisms. These findings further strengthen the notion that the expression of flip/flop splice variants of AMPA-Rs in hippocampal astrocytes is under developmental control.
Ca\textsuperscript{2+} permeability of the glial AMPA receptor channels

The Ca\textsuperscript{2+} permeability of native AMPA-Rs critically depends on the presence of the GluR2 subunit (Geiger et al. 1995). Our previous experiments demonstrated an intermediate Ca\textsuperscript{2+} permeability of AMPA-R channels expressed in hippocampal astrocytes at P12 (Seifert and Steinhäuser 1995). To get information on age-dependent changes in the subunit composition of the glial receptors, the analysis was extended to the cells at P5 and P35. We compared the reversals potentials of receptor currents evoked by kainate in standard bath solution and solutions containing 50 mM CaCl\textsubscript{2}. The I-V relations of the receptor currents were obtained by stepping the membrane between -100 and +70 mV and subtracting the control currents from those activated in the presence of kainate (Fig. 7). No consistent changes in the reversal potentials could be observed within the period analyzed (P5: -28.5 ± 4.8 mV, n = 9; P12: -30.1 ± 6.2 mV, n = 16; P35: -28.7 ± 8.3 mV, n = 8; Fig. 7C). The divalent/monovalent permeability ratio, $P_{Ca}/P_{K}$, was calculated according to the constant-field equation (Mayer and Westbrook 1987), yielding 0.30 ± 0.09 (P5), 0.28 ± 0.09 (P12), and 0.32 ± 0.12 (P35). This invariable Ca\textsuperscript{2+} permeability indicated a rather constant abundance of the GluR2 subunit in the developing hippocampal astrocytes.

DISCUSSION

The present results demonstrate developmental differences in the functional properties of AMPA-Rs in a subpopulation of hippocampal astrocytes, probably reflecting a switch in splice variant expression.

Variation in AMPA receptor composition between P5 and P12

Between P5 and P12, the maximum glutamate currents increased significantly although the amplitudes of equilibrium kainate responses in the glial cells were almost the same for both developmental stages. Recombinant receptor studies have shown that such differences in the maximum responses of glutamate versus kainate can be produced by alternative splicing. Thus compared with the corresponding kainate responses, glutamate currents were much smaller for the flop versions of AMPA-Rs than for the flip (Sommer et al. 1990).

TABLE 2. Changes in the recovery of glutamate responses from cyclothiazide (CTZ) potentiation

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<tr>
<td>Recovery 300 s after CTZ removal (%)</td>
<td>97.2 ± 2.3* (4)</td>
<td>89.3 ± 5.2* (5)</td>
<td>67.8 ± 7.6* (4)</td>
</tr>
<tr>
<td>Recovery 1 s after CTZ removal (%)</td>
<td>4.9 ± 2.7 (4)</td>
<td>6.2 ± 3.5 (5)</td>
<td>3.5 ± 3.8 (4)</td>
</tr>
<tr>
<td>Dissociation time constant $\tau_{fast}$ (s)</td>
<td>14.5 ± 2.9 (4)</td>
<td>10.0 ± 1.0* (5)</td>
<td>12.9 ± 1.9 (3)</td>
</tr>
<tr>
<td>Relative amplitude of $\tau_{fast}$ (%)</td>
<td>57.5 ± 20</td>
<td>44.6 ± 3.5</td>
<td>52.7 ± 14</td>
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<tr>
<td>Dissociation time constant $\tau_{slow}$ (s)</td>
<td>55.5 ± 9.7* (4)</td>
<td>97.8 ± 24.7 (5)</td>
<td>98.4 ± 23.8 (4)</td>
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Values are means ± SD with cell numbers in parentheses. Concentrations of glutamate and CTZ were 1 and 0.1 mM, respectively. P, postnatal days. * Significance of differences.
The selective AMPA-R modulator, CTZ, offers another possibility to distinguish between flip/flop splice variants. It has been shown that receptors carrying the flip forms exhibit a greater sensitivity to CTZ than receptors assembled from flip variants (Johansen et al. 1995; Partin et al. 1994, 1995). Furthermore, the kinetics of recovery from CTZ potentiation differs considerably for both splice variants (Fleck et al. 1996; Partin et al. 1994). In the present study, a significant increase in potentiation of glial kainate responses was found between P5 and P12, which remained constant during further development, and a much faster kinetics of dissociation of CTZ was observed at P5. In conclusion, all the three changes in agonist equilibrium responses, potentiation by CTZ, and recovery from CTZ potentiation observed between P5 and P12 were compatible with the assumption that the astrocytes at P5 express more flip splice variants while the portion of flip is increased with continued maturation.

The observation of a more incomplete desensitization of glutamate- and AMPA-induced currents at P5, however, apparently did not fit into this concept. By combining functional analysis with the single cell RT-PCR technique, we suggested in a previous study that at P12 these cells preferentially express GluR2 flip and GluR4 flip subunits (Seifert et al. 1997). Furthermore, recombinant receptor studies have demonstrated that incomplete desensitization is indicative of the expression of flip splice variants, in particular GluR2 flip (Partin et al. 1994; Sommer et al. 1990). Accordingly, the larger steady-state current at P5 should reflect increased expression of GluR2 flip rather than flop. However it is possible that RNA editing in the arginine/glycine (R/G) site (Lomeli et al. 1994) accounts for the incomplete desensitization in the P5 astrocytes. In the latter study, it was shown that editing at the R/G site of flip and flop versions of the GluR2–4 subunits produces receptor currents with an increased stationary component and that the extent of R/G editing dramatically changes during early postnatal development. In particular, editing of the GluR4 flip reached a maximum at P7 and decreased considerably until P21 in rat brain (Lomeli et al. 1994). It is thus conceivable that, despite an increased contribution of flop versions to the receptor complex, a higher extent of editing at the R/G site of GluR 4 flip produced the larger steady-state component of glutamate and AMPA currents in astrocytes at P5. According to recent findings by Fleck et al. (1996), the absence of a very fast dissociation time constant of CTZ in the glial cells makes the expression of receptor channels assembled exclusively from flop splice variants unlikely.

We cannot exclude that besides differences in splicing, changes in receptor subunit composition affected the properties of AMPA-Rs in the developing glial cells. As an example, homomeric and heteromeric receptors containing the GluR1 subunit displayed a comparatively high kainate affinity (Nakanishi et al. 1990; Stein et al. 1992), whereas variable splicing did not produce different potencies (Partin et al. 1994; Sommer et al. 1990). Hippocampal neurons express receptor complexes with different subunit compositions (Wenthold et al. 1996). Assuming that this also applies to glial cells, the decrease in potency of kainate observed between P5 and P35 could indicate a developmental regulation of the subunit assembly of individual AMPA-R pores.

**Ca**

permeability of AMPA receptor was conserved during development

In situ hybridization and Northern blot analysis suggested that in the hippocampus, the expression of AMPA-Rs is under developmental regulation. The results of these studies are, however, not consistent. For example during the first 2 wk of postnatal development, the relative expression of GluR2, which critically determines the **Ca**

permeability of the receptor channel, was reported to increase (Durand and Zukin 1993), although in two other studies, a decrease was observed (Pellegrini-Giampietro et al. 1992; Standley et al. 1995). Although nonneuronal cells were held responsible

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**TABLE 3.** Desensitization properties of glutamate and AMPA responses in maturing astrocytes

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<tr>
<td><strong>Glutamate response (1 mM)</strong></td>
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<tr>
<td>Desensitization (%)</td>
<td>91.5 ± 8.2* (29)</td>
<td>97.6 ± 2.6 (35)</td>
<td>96.1 ± 4.8 (37)</td>
</tr>
<tr>
<td>Densensitization time constant τ (ms)</td>
<td>7.9 ± 2.3 (32)</td>
<td>8.2 ± 2.4 (32)</td>
<td>8.6 ± 2.4 (60)</td>
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<td><strong>AMPA response (500 μM)</strong></td>
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<tr>
<td>Desensitization (%)</td>
<td>94.0 ± 4.0† (8)</td>
<td>98.6 ± 1.1 (8)</td>
<td>95.0 ± 5.6 (6)</td>
</tr>
<tr>
<td>Densensitization time constant τ (ms)</td>
<td>7.5 ± 2.1 (11)</td>
<td>9.0 ± 2.0 (12)</td>
<td>9.4 ± 1.9 (7)</td>
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</table>

Values are means ± SD with cell numbers in parentheses. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; P, postnatal days. *Significance of differences. †Only the difference between P5 and P12 was statistically significant.
AMPA-Rs indicated a relatively balanced contribution of et al. (1995) and our previous PCR analysis (Seifert et al. the properties of the somatic receptors analyzed here also of receptors located at the distant, nonsomatic membrane of neurons glutamate causes filopodia formation in cultured hippocampal neurons, the functional properties of dendritic areas that particularly are of functional interest because they are in close contact with synapses. Interestingly in hippocampal neurons, the functional properties of dendritic AMPA-Rs were very similar to those found at the soma (Spruston et al. 1995). Future studies have to reveal whether the properties of the somatic receptors analyzed here also match their counterparts at the glial processes.

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FIG. 7. Reversal potentials of kainate-activated mem-
brane currents. A: a cell at p30 was analyzed in standard bath solution (left) and subsequently exposed to a Na⁺-
free solution containing 50 mM CaCl₂ (right). Starting from a holding potential of ~70 mV, membrane was repetitively clamped between −100 and 70 mV for 100 ms, separated by 100-ms intervals. Inset: 1 current family with higher resolution. At holding potential, kainate induced inward currents of 180 pA (Na⁺ solution) and 10 pA (high-Ca²⁺ solution). B: C-V curve of kainate re-
response in high Ca²⁺ was calculated by subtracting current amplitudes at corresponding membrane potentials before addition of agonist (11) from those during kainate application (12). Reversal potential was ~40 mV. C: reversal potentials of kainate responses in 50 mM CaCl₂ solution were plotted as a function of postnatal age. Bars indicate mean and standard deviation for each age group. In all these experiments, K⁺ was replaced by Cs⁺ and tetrodo-
toxin in the pipette solution.


