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AMPA Receptor Development in Rat Telencephalon: [^{3}H]AMPA Binding and Western Blot Studies

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Abstract: Telencephalic membranes from rats of different embryonic (E16, E19) and postnatal (P2, P7, P14, adult) ages were assessed for α-[^{3}H]amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H] AMPA) binding and for immunoreactivity levels of AMPA receptor subunits (GluR1, GluR2/3, and GluR4). In addition, the synaptic markers synaptophysin and $NCAM_{140}$ (a neural cell adhesion molecule isoform) were examined by immunoblot. The density of [^{3}H]AMPA binding sites increased steadily with advancing age. This increase was due mainly to the development of the large low-affinity component (K_D = 400 n*M*) that dominates the [^{3}H]AMPA binding profile of adult rat brain membranes. As resolved by two-site regression analysis, the high-affinity component (K_D = 15 n*M*) of the [^{3}H]AMPA binding increased by approximately twofold from E16 to adult, whereas the low-affinity component increased by 25-fold. Staining for GluR1 and GluR2/3 increased steadily with increasing age at all time points examined; synaptophysin and $NCAM_{140}$ exhibited similar ontogenic immunostaining profiles. GluR4 immunoreactivity was first evident at P14 and increased by adulthood. These results indicate that AMPA receptor density increases steadily during development and that this increase is coincident with the ontogenic expression of other synaptic components. Furthermore, there is a shift toward a preponderance of low-affinity [^{3}H]AMPA binding, which occurs during the period when AMPA receptors are being sorted into postsynaptic regions, suggesting that some element of the postsynaptic membrane environment modulates AMPA receptor properties. **Key Words:** Glutamate receptors—GluR—Ontogeny—Synaptophysin—Neural cell adhesion molecule.
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The subclass of glutamate receptors specifically activated by α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) mediates the majority of fast excitatory transmission in the mammalian brain (Mayer and Westbrook, 1987). These ionotropic receptors are thought to play a key role not only in adult forms of neural plasticity in the mammalian CNS (Kano and Kato, 1987; Lynch et al., 1991), but also in the sculpting of neural connections during development (McDonald and Johnston, 1990). Thus, information concerning the density and properties of AMPA receptors during development is essential to understanding the fundamentals of synapse formation and function in the mammalian CNS.

To date, studies concerning AMPA receptor development have measured either [^{3}H]AMPA binding or AMPA receptor subunit mRNA levels. Analyses of [^{3}H]AMPA binding during rat brain ontogeny consistently reveal increases in binding with advancing age, but comparisons between studies are limited by technical differences. Erdö and Wolff (1990) demonstrated a peak of [^{3}H]AMPA binding in rat visual cortex at postnatal day 6 (P6) followed by a decline to adult levels; because their binding studies were performed in the absence of potassium thiocyanate (KSCN), however, their conclusions presumably extend mostly to high-affinity [^{3}H]AMPA binding sites because low-affinity [^{3}H]AMPA binding sites are not readily detectable in the absence of thiocyanate (Hall et al., 1993). Insel et al. (1990) used autoradiography to determine that binding of a fixed [^{3}H]AMPA concentration (100 n*M*) to many brain areas increases during development. Wahl et al. (1991) also measured [^{3}H]-AMPA binding during development, and at the two time points examined (P4 and P11) a significant increase in receptor density was observed. Although these studies provide a sketch of AMPA receptor development, a comprehensive developmental study of [^{3}H]AMPA binding over many concentrations in the presence of thiocyanate has yet to be performed. It has recently been proposed (Hall et al., 1992) that the well-characterized high- and low-affinity [^{3}H]AMPA binding sites in mammalian brain (Murphy et al., 1987; Olsen et al., 1987; Honoré and Drejer, 1988; Terramani et al., 1988; Hall et al., 1992) may represent extrasynaptic versus synaptic forms of the same receptor. Given this, it would be of particular interest to compare the

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Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; E16, embryonic day 16; GABA, γ-aminobutyric acid; GluR, glutamate receptor; KSCN, potassium thiocyanate; NCAM, neural cell adhesion molecule; P6, postnatal day 6.

ratio of high- and low-affinity [^{3}H]AMPA binding sites during development as the brain moves from a state of relatively few synaptic connections to a state of high synaptic density.

The cloning of the AMPA receptor subunits GluR1–4 (Hollmann et al., 1989; Boulter et al., 1990; Keinänen et al., 1990) has led to in situ hybridization studies measuring the levels of GluR mRNAs; these studies have revealed shifting patterns of GluR mRNAs during development. For example, (1) mRNA levels for the "flip" variants of the AMPA receptor subunits GluR1–3 are fairly uniform between P1 and P15, whereas the mRNA levels for the "flop" forms exhibit dramatic increases during the same time period (Monyer et al., 1991), (2) there is evidence that GluR mRNAs are markedly overexpressed in the neonatal rat relative to the adult (Pellegrini-Giampietro et al., 1991; Standley et al., 1993), and (3) in some brain regions, GluR2 mRNA appears to be less abundant relative to the other subunits in the neonate than in the adult (Pellegrini-Giampietro et al., 1992). These studies, however, do not address the question of whether the levels of the GluR subunit polypeptides parallel their respective mRNA levels throughout development. Polyclonal antibodies that specifically recognize GluR1, GluR2/3, and GluR4 (Rogers et al., 1991; Wenthold et al., 1992) can be used to answer such a question. These antibodies have been used in immunocytochemical studies to demonstrate that staining for the GluR subunits is widespread throughout the rat brain, with regional differences between subunits (Rogers et al., 1991; Petralia and Wenthold, 1992; Martin et al., 1993; Molnar et al., 1993). Given that the GluR subunits have been shown to assemble into heteromeric receptors with different properties dependent on subunit composition (Hollmann et al., 1991; Verdoorn et al., 1991), the observation that there are differences in the expression patterns of the GluR subunits leads naturally to speculation that such differences might have significant functional consequences. In light of the suggestive data from the aforementioned in situ hybridization studies, it would clearly be of interest to measure the levels of GluR immunoreactivity during development and to determine whether there are any accompanying changes in ligand binding properties. In the present study, we quantified changes in AMPA receptor density and binding properties in the developing rat telencephalon (embryonic days E16 and E19 and P2, P7, P14, and adult) by analyzing [^{3}H]AMPA binding over a wide range of concentrations and by performing western blot analyses for GluR1, GluR2/3, and GluR4. We also performed western blots measuring synaptophysin and neural cell adhesion molecule isoform $NCAM_{140}$ immunoreactivity levels to compare AMPA receptor development with ontogenic changes in other synaptic components.

MATERIALS AND METHODS

Preparation of membranes

Telencephalic tissue was collected from Sprague–Dawley rats of the following varying embryonic and postnatal ages: 16 (E16) and 19 (E19) days after conception and 2 (P2), 7 (P7), 14 (P14), and 60–90 (adult) days after birth. Groups of eight to 12 embryos were combined to form each embryonic sample, groups of two to four pups were combined to form the P2 and P7 samples, and each P14 and adult sample was prepared from a single brain. The brains were homogenized in 0.32 *M* sucrose, 1 m*M* EGTA (20 ml/brain) using a glass–Teflon homogenizer. The homogenate was centrifuged at 800 *g* for 10 min and the supernatant recentrifuged at 48,000 *g* for 30 min. The resultant membrane pellet (P_2) was lysed by resuspending it in 20 ml of distilled H_2O containing 1 m*M* EGTA followed by a 20-min incubation on ice and centrifugation at 48,000 *g* for 30 min. The lysis and centrifugation cycle was repeated and the pellet resuspended in the assay buffer (100 m*M* Tris-acetate, 50 μ*M* EGTA, pH 7.2) to ~1 mg/ml; this sample is referred to as "membranes." All procedures for membrane preparation were performed at 0–4°C. Samples were frozen at −80°C for up to 2 weeks until use. The same samples were used for the binding as for the western blots; for the postnatal time points, however, additional western blots were performed on samples on which binding had not been measured.

Receptor binding assays

[^{3}H]AMPA binding was studied using the centrifugation method. Typically, 50–100 μg of protein in a final volume of 100 μl was equilibrated with [^{3}H]AMPA for 40–60 min at 0°C in the presence of 50 m*M* KSCN. To determine binding constants, AMPA concentrations were chosen between 2 and 1,500 n*M*. Concentrations between 2 and 50 n*M* were measured by increasing the concentration of radiolabeled AMPA, whereas those >50 n*M* were reached by adding unlabeled AMPA. Nonspecific binding was defined as that which occurred in the presence of 2.5 m*M* unlabeled L-glutamate. Samples were centrifuged at 48,000 *g* (4°C) for 20 min, then placed back in the ice-water bath. The supernatants were aspirated within 10–20 min and the pellets superficially rinsed with 0.4 ml of ice-cold assay buffer and then dissolved in 10 μl Beckman tissue solubilizer. Radiolabel content was assayed by liquid scintillation spectroscopy with a counting efficiency of 0.40 in 1 ml of aqueous counting scintillant. Nonspecific binding increased from ~15 to 75% of the total binding as AMPA concentration increased from 2 to 1,500 n*M*. One- and two-site analyses of binding data were performed by nonlinear regression using the Inplot program by GraphPad Software, Inc.; comparisons for one- versus two-site fits were made using the F^2 test ($p < 0.05$). [^{3}H]AMPA (46.1 Ci/mmol) was obtained from NEN/Du Pont and AMPA was obtained from Tocris.

Western blot analysis

Membrane samples (40–80 μg of protein) were treated with 2.5% (wt/vol) sodium dodecyl sulfate in the presence of 3% (vol/vol) 2-mercaptoethanol at 100°C for 5 min, then subjected to polyacrylamide gel electrophoresis based on the method developed by Laemmli (1970). Linear acrylamide gradient gels (3–17%, wt/vol) were used to separate proteins that then were transferred to nitrocellulose (0.2-μm pore size) for 1 h with the Trans-Blot system from Bio-Rad Laboratories. Incubation of the nitrocellulose with rabbit antibodies against individual GluR subunits (Hennegriff et al., 1992; note, antibodies were developed with a procedure nearly identical to that of Wenthold et al., 1992) diluted 1:900, monoclonal anti-synaptophysin (Boehringer Mannheim) diluted 1:1,000, or rabbit anti-mouse NCAMs (cour-

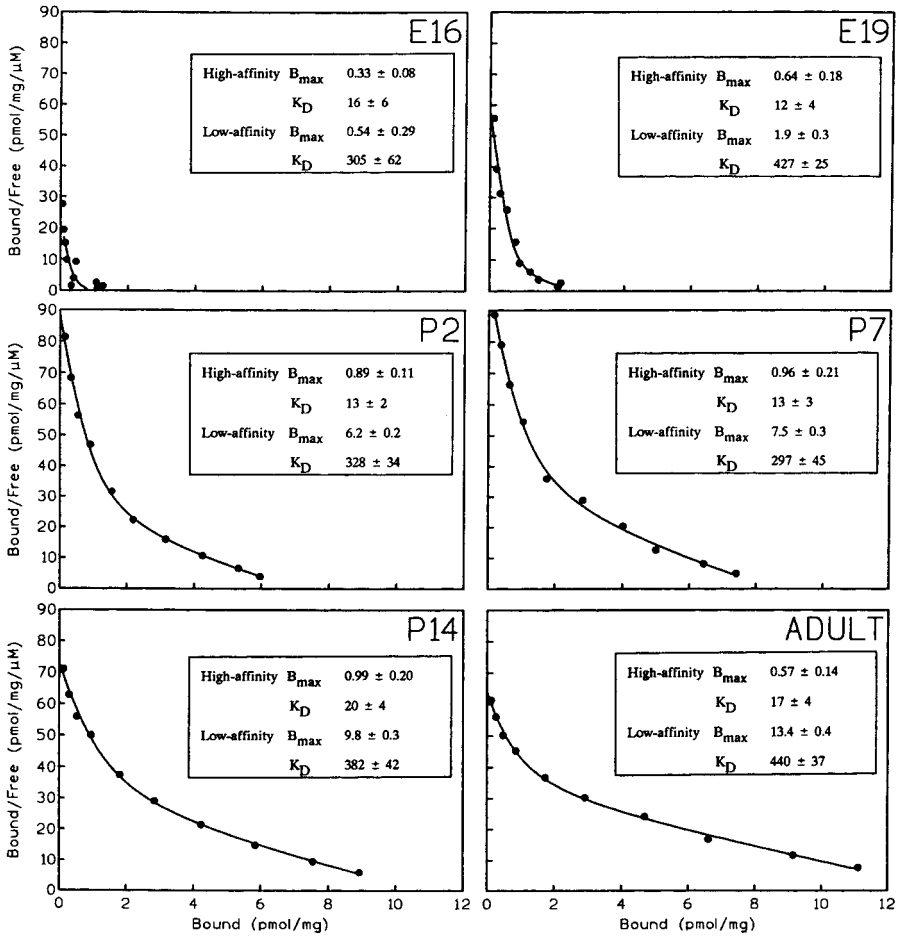

FIG. 1. Scatchard plots of [^{3}H]-AMPA binding to telencephalic membranes prepared from rats of different ages. Each point represents the mean of three independent determinations, each performed in triplicate; error bars (SEM < 10% of mean value) have been omitted for clarity. B_{max} and K_D estimates (with SEM) for two-site linear-regression analyses of these data are provided in the insets. The units for the inset values are picomoles per milligram (B_{max}) and nanomolar concentrations (K_D). A graphic representation of the B_{max} estimates is shown in Fig. 2.

tesy of Dr. Ben Murray, Department of Developmental and Cell Biology, University of California, Irvine, CA, U.S.A.) diluted 1:1,000 in Tris-buffered saline, pH 7.4, with 0.1% (vol/vol) Tween 20 and 1.5% (wt/vol) nonfat dry milk was performed at 4°C with agitation for 12–16 h. Secondary antibody incubation and color development used goat anti-IgG alkaline phosphatase conjugates and a 5-bromochloro-3-indolyl phosphate and nitro blue tetrazolium substrate system. Color development of immunoreactive bands was terminated well before maximal intensity was reached, to avoid saturation and allow comparative studies within each blot. The relative optical densities and image areas of the labeled antigens were quantitatively compared within single immunoblots using a computerized image analysis system; the specific immunoreactivity [(density − background) × area] for each blot lane was determined from these values. Calibration of immunoblots using prestained protein molecular weight standards allowed the determination of the M_r for pertinent species.

To test if GluR immunostaining on blots is linear within the protein range examined, GluR immunoreactivity for samples containing 5–100 μg of protein of the adult telencephalon samples (n = 14–21) was measured and plotted versus protein. These graphs were linear between ~8 μg and ~100 μg for anti-GluR1 (r = 0.96) and anti-GluR2/3 (r = 0.98); samples with <8 μg of protein gave variable or undetectable immunoreactivity values. For both anti-GluR4 and the monoclonal anti-synaptophysin antibody used in this study, staining was not consistently detectable for protein values <25 μg of adult telencephalon but was linear for protein values between 25 and 100 μg (r = 0.91 and 0.99, respectively).

RESULTS

[^{3}H]AMPA binding studies indicate changes in AMPA receptor density and properties during development

Binding of 2–1,500 nM [^{3}H]AMPA to rat telencephalon membranes at six developmental stages was measured. The saturation curves for bound [^{3}H]-AMPA were better fit in all cases by assuming two binding sites instead of one (F^2 test, $p < 0.05$); thus, these curves were resolved into high- and low-affinity components. Scatchard plots of the data are shown in Fig. 1 along with transformed best-fit curves; the estimates for the high- and low-affinity components of the graphs are presented as insets to Fig. 1. The total B_{max} values (high- and low-affinity components combined) increased steadily with advancing age, but there

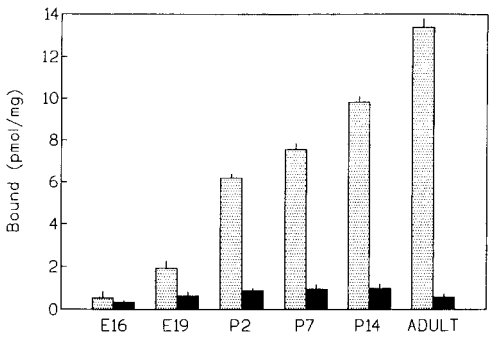

FIG. 2. Plot of B_{max} estimates for low-affinity binding (speckled bars) versus high-affinity binding (dark bars) during rat telencephalic development. B_{max} estimates were determined from two-site regression analyses of the data shown in Fig. 1. Error bars indicate the SEM.

was a dramatic difference in the rate of increase between the high- and low-affinity sites. The B_{max} estimates for the high-affinity component increased by approximately threefold from E16 to P14, then decreased by 40% to the adult value; meanwhile, B_{max} estimates for the low-affinity component increased by 25-fold from E16 to adult. Figure 2 demonstrates these changes graphically by plotting the B_{max} values of low-affinity and high-affinity binding versus age. In contrast to the changing B_{max} values, the K_D estimates for the high- and low-affinity components were similar across all ages; the K_D for the high-affinity component ranged from 12 to 20 nM, whereas the K_D for the low-affinity component had a range of 300–440 nM.

AMPA receptor subunits parallel synaptic markers during development

The telencephalic membrane samples from above were analyzed by immunoblot to quantitate (per milligram of protein) the relative immunoreactivity levels for the AMPA receptor subunits GluR1, GluR2/3, and GluR4 and for the synaptic markers synaptophysin and $NCAM_{140}$. Figure 3 shows typical western blots of the samples stained with antibodies to GluR1 and GluR2/3, and the mean GluR immunoreactivities across age groups are shown in the top panel of Fig. 4. The GluR antibodies labeled a single antigen of ~105 kDa. At the earliest developmental stage tested (E16), no staining for any of the GluR antigens was evident; at E19, light staining was evident for only GluR1. Subsequently, staining for GluR1 and GluR2/3 increased steadily throughout postnatal development, whereas the GluR4 antigen was not evident until P14. Staining for all three anti-GluR antibodies was highest in the adult samples.

Inserts in Fig. 4 show typical immunoblots of telencephalic samples stained with antibodies to the presynaptic marker synaptophysin (middle panel) and to NCAM isoforms of 180, 140, and 120 kDa (bottom panel). The profiles for synaptophysin and $NCAM_{140}$ immunoreactivity levels across age groups (Fig. 4, middle and bottom panels, respectively) resembled those for the GluR subunits closely (Fig. 4, top panel). The two synaptic markers were evident at E19 and increased throughout the postnatal time points, with the exception that they did not continue to increase from P14 to adult as did the GluR subunits. $NCAM_{140}$ was chosen for analysis over $NCAM_{180}$ as the latter is known to undergo a pronounced change in glycosylation state during development (Pollerberg et al., 1985) and is therefore difficult to analyze quantitatively; this conversion is evident in the anti-NCAM blots in Fig. 4 (bottom panel). $NCAM_{120}$ was only faintly detected in P7 to adult samples.

DISCUSSION

Both the binding data and the western blots demonstrate a large and steady increase in AMPA receptor density during telencephalic development; the binding data indicate that this increase is accompanied by a shift toward low-affinity [^{3}H]AMPA binding. A similar shift in binding properties has recently been reported for ifenprodil inhibition of ^{125}I-MK-801 binding to the NMDA receptor (Williams et al., 1993). This change in NMDA receptor pharmacological properties is most likely due to a developmental switch in subunit composition of the receptor (Watanabe et al., 1992; Williams et al., 1993). γ-Aminobutyric $acid_A$ ($GABA_A$) receptors also exhibit a shift toward lower affinity for agonists during development (Meier and Schousboe, 1982) as well as changes in affinity for benzodiazepines (Lippa et al., 1981; Chisolm et al., 1983); these ontogenic changes in pharmacology are thought to be due to the shifting subunit composition of the receptor (Gambarana et al., 1991; Zhang et al., 1991; Laurie et al., 1992). Another ionotropic receptor, the nicotinic acetylcholine receptor at the neuro-

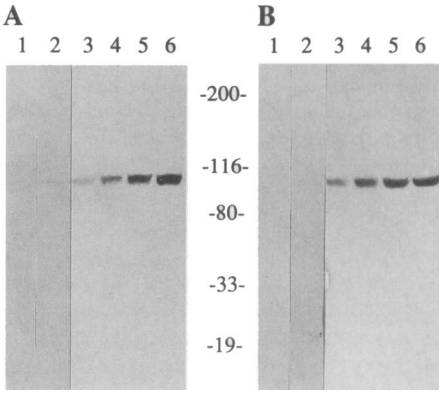

FIG. 3. Immunochemical analyses of telencephalic AMPA receptor subunits during rat development. Equal amounts of protein (30 μg) from E16, E19, P2, P7, P14, and adult membrane samples (blot lanes 1–6, respectively) were subjected to electrophoresis, blotted to nitrocellulose, and stained with anti-GluR1 (**A**) and anti-GluR2/3 (**B**). The positions of 19–200-kDa molecular mass standards are indicated.

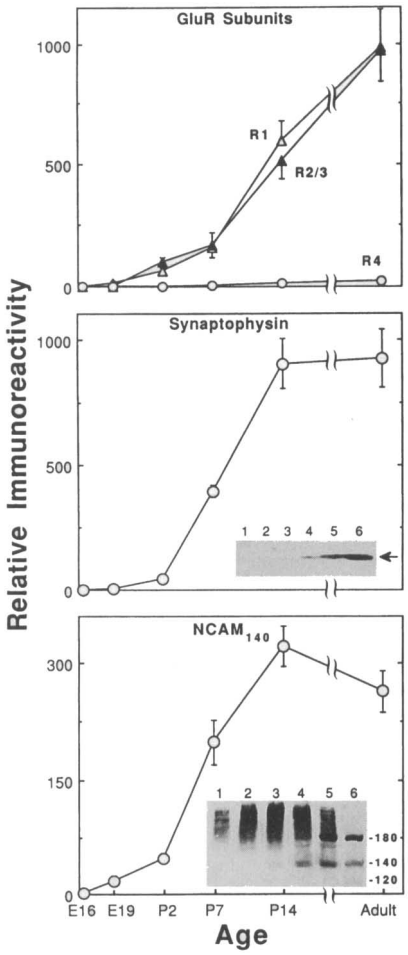

FIG. 4. Changes in AMPA receptor subunits and synaptic markers during telencephalic development. Equal aliquots of samples from E16 (n = 3), E19 (n = 3), P2 (n = 6), P7 (n = 6), P14 (n = 6), and adult (n = 5) membranes were analyzed by immunoblot (see Materials and Methods and Fig. 3) to determine the relative immunoreactivity levels for the AMPA receptor subunits GluR1, GluR2/3, and GluR4 (**top**) and for the synaptic markers synaptophysin (**middle**) and $NCAM_{140}$ (**bottom**). Each datum represents the mean specific immunoreactivity of the antigen [(density − background) × area] ± SEM. Typical immunoblot results obtained with anti-synaptophysin (insert in middle panel) and anti-NCAMs (insert in bottom panel) are shown; telencephalic samples in lanes 1–6 are from E16, E19, P2, P7, P14, and adult membranes, respectively. The electrophoretic positions of synaptophysin (arrow), $NCAM_{180}$, $NCAM_{140}$, and $NCAM_{120}$ are indicated.

muscular junction, also undergoes changes in subunit configuration and receptor properties during development (Mishina et al., 1986). Thus, it is plausible that our observed shift in the binding properties of AMPA receptors during development might be due to a change in the subunit composition of the receptor. Although we cannot rule this out, there are two observations which are inconsistent with this possibility. First, our western blot data show nearly identical increases for both GluR1 and GluR2/3 staining. This suggests that, although subtle developmental changes in relative GluR mRNA levels may occur in selected forebrain structures (Monyer et al., 1991; Pellegrini-Giampietro et al., 1991, 1992), the developing telencephalon as a whole exhibits a steady rise in the density of GluR1–3 subunits. Second, even if there were a change in subunit composition of the AMPA receptor with development, it appears unlikely that this could explain the observed pharmacological shift; it has been shown that recombinantly expressed GluR1–4 subunits, both flip and flop forms as well as various subunit combinations, have similar affinities for [^{3}H]AMPA (Rasmussen et al., 1992). It is interesting that the K_D values (8–27 nM) for [^{3}H]AMPA binding to these receptor subunits expressed in kidney cells are similar to the values that we and others have determined for the high-affinity component for [^{3}H]AMPA binding to mammalian brain tissue (Murphy et al., 1987; Olsen et al., 1987; Honoré and Drejer, 1988; Terramani et al., 1988; Hall et al., 1992). The suggestion has been made (Hall et al., 1992) that these high-affinity binding sites represent AMPA receptors that do not reside in a synaptic membrane environment, as may be the case for newly created neuronal AMPA receptors and would clearly be the case for receptors expressed in kidney cells.

One hypothesis concerning the locus of expression for long-term potentiation, a long-lasting form of synaptic plasticity, is that it may be a change in AMPA receptor properties mediated by a stable factor in the postsynaptic membrane (for a review, see Lynch et al., 1991). However, it is not known whether the properties of the AMPA receptor, or indeed any ionotropic neurotransmitter receptor, can be influenced strongly by interaction with the surrounding membrane environment. There is evidence that such modulatory associations may occur; i.e., patch clamp experiments have revealed that NMDA receptor properties may be altered by cytoskeletal interactions (Rosenmund and Westbrook, 1993), and radiation inactivation experiments have suggested that AMPA receptors associate with a modulatory protein (Honoré and Nielsen, 1985; Henley, 1993). Craig et al. (1993) have shown that during the first week after being cultured from E18 rats, hippocampal neurons begin segregating GluR subunits to somatodendritic regions. This distribution of receptors into protosynaptic areas would be coincident with the beginning of the large increase in low-affinity [^{3}H]AMPA binding seen in the present study. It is thus possible that as AMPA receptor subunits are sorted into developing postsynaptic membranes, receptor properties become modulated by a factor or group of factors in the surrounding cytoskeleton or postsynaptic density.

In the present study, we observed [^{3}H]AMPA binding even before any staining for GluR subunits was evident. It appears unlikely that [^{3}H]AMPA binds to sites in the brain other than the GluR subunits, because the binding of [^{3}H]AMPA overlaps extremely well with the localization of GluR subunits by immunocyto-

chemistry [compare Monaghan et al. (1984) with Petralia and Wenthold (1992) and Martin et al. (1993)]. The likely explanation for the discrepancy between the binding data and the western blot data is that the [^{3}H]AMPA binding assay is more sensitive in detecting the presence of AMPA receptor subunits than are immunochemical techniques. (The thresholds of detection for the antibodies used in this study are described in Materials and Methods.) The difference in sensitivity between the binding assay and the blots also explains the observation that the increase in the relative staining of GluR1 and GluR2/3 in the adult is some 10 times higher than at P2, whereas the increase in total binding at the same two time points is only a factor of two. Given the limitations in sensitivity of the anti-GluR antibodies, it is likely that the total B_{max} values from the binding studies represent a more accurate depiction of the relative time course of AMPA receptor development than do the western blot studies.

Data in this report reveal that [^{3}H]AMPA binding reaches a level that is more than one-half of the adult value by P7, a time point that studies of rat telencephalic development designate as the onset of intense synaptogenesis. It has been shown in rat parietal cortex (Aghajanian and Bloom, 1967), rat visual cortex (Blue and Parnavelas, 1983), and dentate gyrus (Crain et al., 1973) that the bulk of synaptogenesis does not occur until after the first postnatal week; in these studies, the density of synapses per unit area is 10–15 times higher in the adult than at the end of the first postnatal week. Because the density of AMPA receptors increases less than twofold during the same period, it is clear that developing neurons possess a high density of AMPA receptors even before the formation of mature synaptic contacts. This is consistent with data from the neuromuscular junction, where developing myotubes reach a high density of acetylcholine receptors even before the arrival of ingrowing motor axons (see Hall and Sanes, 1993, for a review). The presence of a fairly high level of low-affinity [^{3}H]AMPA binding at ages before P7, and therefore before the bulk of synaptogenesis, may appear inconsistent with our hypothesis described above that the low-affinity binding represents binding to AMPA receptors in "synaptic" environments. However, several studies of rodent telencephalic development have demonstrated precocious postsynaptic specializations that may precede the formation of complete synaptic complexes (Hinds and Hinds, 1976; Bähr and Wolff, 1985; for a review, see Vaughn, 1989); thus, neurotransmitter receptors may be inserted into a synapselike environment even before the formation of mature synaptic contacts. The developmental profiles for the presynaptic marker synaptophysin (Jahn et al., 1985; Wiedenmann and Franke, 1985) and the pre- and postsynaptic marker $NCAM_{140}$ (Persohn et al., 1989) in this study are consistent with the notion that both pre- and postsynaptic elements are forming before the establishment of mature synaptic complexes. Furthermore, the observation that the GluR subunits and synaptic markers have similar developmental profiles suggests a tightly controlled orchestration of the expression of synaptic components. The observed increase in synaptophysin immunoreactivity confirms the results of Knaus et al. (1986), who reported a nearly identical postnatal increase in synaptophysin staining in mouse cerebrum.

It has previously been reported (Pellegrini-Giampietro et al., 1991; Standley et al., 1993) that levels for the GluR mRNAs exhibit a transient overexpression in the first and second postnatal weeks and then decrease significantly to adult levels. Our western blot data, however, indicate that GluR protein levels do not follow the ontogenic patterns for the respective mRNAs. As shown in Figs. 3 and 4, all three anti-GluR antibodies showed their highest levels of staining in the adult tissue. This apparent uncoupling between mRNA and protein levels may be the result of differences in tissue preparation; in situ hybridization procedures use intact brain slices, whereas we examined homogenized membranes that had been lysed and washed. Technical differences aside, the relationship of mRNA levels to protein levels depends on several factors, including the relative rates of translation, proteolysis, and mRNA degradation. If any of these factors change during development, discrepancies in the ratio of mRNA to protein might result.

In summary, we have demonstrated an increase in [^{3}H]AMPA binding B_{max} and a concomitant increase in the density of GluR subunits during rat telencephalic development. The ratio of low- to high-affinity [^{3}H]-AMPA binding sites increased significantly with advancing age. Because the shift toward low-affinity binding occurred during the period when AMPA receptors are being sorted into postsynaptic regions, and did not coincide with any apparent change in the ratio of AMPA receptor subunits, a possible interpretation of the data is that the shift toward low-affinity binding was due to the insertion of AMPA receptors into developing postsynaptic membranes. If this interpretation is correct, it would represent evidence that the properties of AMPA receptors can be modulated by components of the synaptic environment.

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