Molecular distinction of three $N$-methyl-$d$-aspartate-receptor subtypes \textit{in situ} and developmental receptor maturation demonstrated with the photoaffinity ligand $^{125}$I-labeled CGP 55802A

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ABSTRACT Activation of $N$-methyl-$d$-aspartate (NMDA) receptors is essential for synaptic plasticity in the central nervous system and contributes to neuronal death under various pathological conditions. Although several subunits have been cloned, the structure of NMDA receptors \textit{in situ} is unresolved. By using a photoreactive antagonist with nanomolar affinity to the NMDA-binding site, three types of receptors were differentiated by their pattern of photoaffinity-labeled subunits. In adult brain, a protein of 175-kDa was photoreactive that displayed a profile of ligand binding and autoradiographical distribution corresponding to NMDA receptors. In contrast, in early postnatal brain, proteins of both 175 kDa and 115 kDa were photolabeled. This labeling pattern is switched to that of adult brain around postnatal day 10, pointing to a structural maturation of NMDA receptors. A third type of receptor could be identified in cerebellar granule cell cultures, where NMDA receptors mediate trophic effects and photolabeling was exclusively targeted to a 115-kDa protein. To identify the proteins labeled \textit{in situ}, recombinant receptors were subjected to photolabeling. When the NR1 subunit was coexpressed with either the NR2A, NR2B, or NR2C subunit, only the combination of NR1/NR2A was photoreactive. Both the NR1 and NR2A subunits were photolabeled, corresponding in size to the proteins labeled \textit{in situ}. However, the lack of subunit-selectivity in photolabeling the NR1/NR2A combination suggests the presence of additional receptor components \textit{in situ} to explain the subunit-selective photoreactivity in adult brain (175 kDa) and in cerebellar granule cells (115 kDa). The subunit combination NR1/NR2A by itself appears insufficient to describe a major population of NMDA receptors, in particular, in adult brain.

In the neurotransmission of excitatory amino acids in the central nervous system, $N$-methyl-$d$-aspartate (NMDA) receptors are essential for activity-dependent synaptic plasticity, such as long-term potentiation or long-term depression, that underlie learning and memory functions (1–4). In addition, NMDA receptors are thought to be involved in the pathophysiology of several neurological disorders such as stroke, epilepsy, or Parkinson disease (5–8). Several constituent subunits of the NMDA receptor have recently been cloned (9–13), and functional studies suggest that native NMDA receptors are composed of the NR1 subunit and at least one member of the NR2 subunit family (11, 12). However, the full structural organization of the receptor complex \textit{in situ} is not understood. The biochemical identification of the receptor was largely restricted to radioligand-binding studies (14, 15), and purification of the receptor has met with only limited success (16, 17). To probe the protein structure of native NMDA receptors we have developed a photoaffinity ligand, which interacts with nanomolar affinity with the binding site for NMDA agonists and competitive antagonists. By using this tool, the structural elements forming the agonist/antagonist-binding site of NMDA receptors can be identified. In addition, because NMDA-receptor subtypes are expected to differ in subunit composition, the pattern of photoreactive proteins may serve as a marker for particular receptor subtypes \textit{in situ}. Finally, the photolabeling technique can be used to monitor the expression of NMDA-receptor subunits under different physiological and pathological conditions.

METHODS

The photoaffinity ligand $^{125}$I-labeled CGP 55802A (specific radioactivity 1150 Ci/mmol; 1 Ci = 37 GBq) was synthesized as described elsewhere (R.H., H.A., and C.A., unpublished work). For radioligand binding, rat whole-brain membranes were washed with 100 mM Tris acetate, pH 8.0/0.03% (wt/vol) Triton X-100 and the same buffer without detergent. Aliquots (250 μg of protein per assay) were incubated with 1.0 nM $^{125}$I-labeled CGP 55802A for 30 min at room temperature in 100 mM Tris acetate, pH 8.0, with the level of nonspecific binding (18% of total binding) being determined in the presence of 2 mM NMDA or 1 mM glutamate. Rapid filtration through GF/C filters (Whatman) was followed by washing twice with 4 ml of ice-cold incubation buffer. For photoaffinity labeling (50 μg of protein per assay) the 30-min incubation was followed by irradiation with UV light (355 nm; 36-W TLD lamp, Philips) for 5 min at a distance of 3 cm. After being washed with 100 mM Tris acetate, pH 8.0, at 4°C by centrifugation, the samples were subjected to 6.5% SDS/PAGE followed by autoradiography (Kodak X-Omat AR film for 2–5 days) and densitometric analysis of the autoradiograms (Shimadzu, Kyoto, CS-930). The pattern of photolabeled proteins was the same when Triton X-100 was omitted from the membrane preparation.

For autoradiography, cryostat sections, kept in 100 mM Tris acetate, pH 8.0, for 40 min at room temperature, were incubated with 1 nM $^{125}$I-labeled CGP 55802A as described above for membranes. For reversible binding the sections were subsequently washed for 20 min in ice-cold incubation buffer (three buffer changes). In the case of photoaffinity labeling sections were exposed to 355-nm light for 5 min, followed by washing for 10 min at room temperature in incubation buffer containing 1 mM glutamate (three buffer changes). The sections were air-dried before exposure to film.

Electrophysiological studies were done on transverse hippocampal slices (400 μm) prepared from male Tif:RAI (SPF).

Abbreviations: NMDA, $N$-methyl-$d$-aspartate; Pn, postnatal day n.
rats (150–200 g), essentially as described (18). Cerebellar granule cells from dissociated cerebella of P7 rats were cultured for 9 days, as described (19).

Transfection of 293 cells (ATCC CRL 1573) with cDNAs encoding NMDA-receptor subunits was done as described by Monyer et al. (11). After 48 hr, the cells were harvested in phosphate-buffered saline, pH 7.4. To determine the presence of glutamate-binding sites [3H]glutamate binding was done on well-washed membranes of 293 cells by filtration and centrifugation binding procedures, using excess nonradioactive glutamate to define the level of nonspecific binding. Significant levels of specific binding were obtained for all homo- and heteromeric recombinant NMDA receptors (D.L. and P.S., unpublished work).

RESULTS

Reversible Receptor Interactions. To test the selectivity of the radioligand 125I-labeled CGP 55802A (Fig. 1), reversible binding experiments were performed on rat brain membranes. Saturation isotherms (1–100 nM of radioligand) revealed an interaction of two binding sites with distinct affinity constants $K_{D1} = 6.6 \pm 2.8 \text{nM}$ and $K_{D2} = 49 \pm 14 \text{nM}$. The corresponding numbers of binding sites were $B_{max1} = 450 \pm 180 \text{fmol/mg}$ of protein and $B_{max2} = 980 \pm 240 \text{fmol/mg}$ of protein. Two binding sites for 125I-labeled CGP 55802A were not found only in adult brain but also during postnatal development, as shown for brain membranes from postnatal day (P) P0, P10, and P30 (P0: $K_{D1} = 8.2 \pm 2.0 \text{nM}$ and $K_{D2} = 158 \pm 93 \text{nM}$; P10: $7.5 \pm 2.5 \text{nM}$ and $54 \pm 17 \text{nM}$; P30: $5.3 \pm 2.3 \text{nM}$ and $31 \pm 13 \text{nM}$).

The profile of drugs by which 125I-labeled CGP 55802A could be displaced from brain membranes suggests that this ligand interacts with the NMDA-agonist/antagonist site (Table 1). It is particularly noteworthy that the binding site displayed stereoselectivity with an $\approx 100$-fold difference in potency between the pharmacologically active enantiomer of an NMDA antagonist (CGP 40116; ref. 20) and the inactive enantiomer (CGP 40117). The lack of displacing potency of kainate, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and MK801 further supports the selectivity of the radioligand.

To determine whether CGP 55802A acts as an agonist or antagonist at the NMDA receptor, its effect on synaptically evoked responses in hippocampal slices was evaluated in Mg2+-free medium by recording field potentials in the CA1 region after stimulation of the Schaffer collaterals (Fig. 2). The initial response, which is known to be mediated by non-NMDA receptors (4) remained unaffected in the presence of CGP 55802A (up to 100 $\mu$M), whereas the subsequent multiple spikes that are mediated via NMDA receptors (4) were potently reduced. At 1 $\mu$M concentration the compound drastically reduced the amplitude to $\approx 10\%$ of control values (Fig. 2) with the threshold concentration being <0.1 $\mu$M. These findings characterize CGP 55802A as a selective and potent NMDA antagonist.

Photoaffinity Labelling. To identify the constituent protein to which 125I-labeled CGP 55802A is bound at the NMDA receptor, the ligand was photoactivated. Incubation of 125I-labeled CGP 55802A (1 nM) for 30 min with membranes from adult brain was followed by irradiation with UV light at 355 nm and analysis by SDS/PAGE. A single protein band of 175 kDa was specifically labeled. Nonspecific photolabeling done in the presence of 2 mM NMDA amounted to <3% of total labeling. Specific photolabeling of a protein band of 175 kDa was observed also in bovine, porcine, chicken, monkey, and human brain membranes. The photolabeled protein is a glycoprotein, as shown by a reduction in size to 165 kDa after deglycosylation of photolabeled rat brain membranes with N-glycosidase F. In addition to the 175-kDa band a faint specifically labeled band at 115 kDa was apparent after gel overexposure. This band showed a reduction to 105 kDa after deglycosylation.

For reversible binding whole-brain membranes were incubated with 1 nM 125I-labeled CGP 55802A in the presence of various concentrations of displacing agents. For photoaffinity labeling the incubation was followed by irradiation for 5 min at 355 nm. After SDS/PAGE the incorporation of radioactivity in the 175-kDa band was analyzed densitometrically. IC50 values were determined from plots of the OD values. ACBD, trans-1-amino-cyclobutane-1,3-dicarboxylic acid. CGP 40116 and CGP 40117 are the pharmacologically active and inactive enantiomers of the racemic NMDA antagonist CGP 37849 (20). AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APS, 2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; MK801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate.

**Table 1. Inhibition of reversible binding and photoaffinity labeling of 125I-labeled CGP 55802A**

<table>
<thead>
<tr>
<th>Displacer</th>
<th>Reversible binding IC50, nM</th>
<th>Irreversible photolabeling IC50, nM</th>
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<tbody>
<tr>
<td>ACBD</td>
<td>251 ± 12</td>
<td>92 ± 9</td>
</tr>
<tr>
<td>Glutamate</td>
<td>280 ± 40</td>
<td>430 ± 130</td>
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<tr>
<td>NMDA</td>
<td>1,460 ± 190</td>
<td>12,700 ± 1,600</td>
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<tr>
<td>CGP 40116</td>
<td>15 ± 8</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>CGP 37849</td>
<td>47 ± 14</td>
<td>20 ± 9</td>
</tr>
<tr>
<td>APS</td>
<td>530 ± 290</td>
<td>700 ± 450</td>
</tr>
<tr>
<td>CGP 40117</td>
<td>1,930 ± 700</td>
<td>2,250 ± 750</td>
</tr>
<tr>
<td>Kainic acid</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>AMPA</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
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<tr>
<td>MK801</td>
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<tr>
<td>Glycine</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>7-Chlorokynurenic acid</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>CNQX</td>
<td>&gt;10,000</td>
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For reversible binding whole-brain membranes were incubated with 1 nM 125I-labeled CGP 55802A in the presence of various concentrations of displacing agents. For photoaffinity labeling the incubation was followed by irradiation for 5 min at 355 nm. After SDS/PAGE the incorporation of radioactivity in the 175-kDa band was analyzed densitometrically. IC50 values were determined from plots of the OD values. ACBD, trans-1-amino-cyclobutane-1,3-dicarboxylic acid. CGP 40116 and CGP 40117 are the pharmacologically active and inactive enantiomers of the racemic NMDA antagonist CGP 37849 (20). AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APS, 2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; MK801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate.

**Fig. 1.** Structure of the photoaffinity ligand 125I-labeled CGP 55802A. CGP 55802A is the sodium salt of (E)-2-amino-10-[(4-azido-2-hydroxy-3-isobenzyloxy)laminio]-4-phosphonomethyl-dec-3-enoic acid. The position of 125I is indicated by *.

**Fig. 2.** Antagonism by CGP 55802A of the NMDA-receptor-mediated synaptic responses in hippocampal slices. Field potentials were recorded in the CA1 region after stimulation of the Schaffer collaterals. The recordings, done in Mg2+-free medium, are preceded by a calibration pulse.
To assess whether the photolabeled protein of 175 kDa was part of the NMDA receptor the potency of various ligands in inhibiting the photoaffinity reaction was tested. The incorporation of radioactivity in the 175-kDa band was evaluated by scanning the autoradiograms. The ligand concentrations required for half-maximal inhibition of the photoreaction were very similar to those found for the inhibition of reversible binding of $^{125}$I-labeled CGP 55802A apart from a lower potency of NMDA (Table 1). In addition, the stereoselectivity demonstrated for the active and inactive enantiomers CGP 40116 and CGP 40117 was retained for the photolabeled site (Table 1). These results suggest that the binding site for NMDA-receptor agonists and competitive antagonists in adult brain is associated with a receptor constituent of 175 kDa.

The existence in brain of pharmacologically different NMDA-receptor subtypes has been reported (14, 15, 21-23). Photolabeling of membranes from various brain areas consistently yielded a single protein band of 175 kDa (Fig. 3). However, the intensity of the photolabeling showed regional variations corresponding to the regionally different density of NMDA receptors. Specific photolabeling was strongest in hippocampus, followed by cerebral cortex, striatum, thalamus/hypothalamus, olfactory bulb, brainstem, and cerebellum (Fig. 3). No major regional distinction in the profile of ligand affinities was apparent because CGP 40116, 2-amino-5-phosphonoopentanoic acid (AP5), and NMDA showed comparable potencies in inhibiting the photoreaction in different brain areas; (their IC$_{50}$ values in cerebral cortex were 11 ± 5 nM, 820 ± 110 nM, 18 ± 1 μM; in hippocampus 14 ± 4 nM, 700 ± 70 nM, 24 ± 8 μM; in thalamus/hypothalamus 14 ± 6 nM, 660 ± 310 nM, 23 ± 5 μM; in cerebellum 21 ± 12 nM, 710 ± 350 nM, 16 ± 4 μM, respectively). These results suggest a wide distribution of the photolabeled NMDA-receptor constituent with no major regional distinction in ligand affinity.

To visualize the distribution of the photolabeled receptor constituent at higher resolution, NMDA receptors were mapped autoradiographically on brain slices by using $^{125}$I-labeled CGP 55802A, either as reversible ligand or as photoaffinity label. The autoradiographic distribution of reversibly or irreversibly bound $^{125}$I-labeled CGP 55802A was virtually identical (Fig. 4). Highest labeling was apparent in hippocampus, cerebral cortex, and thalamus; lower labeling was present in olfactory bulb, striatum, cerebellum (granule cell layer); and only faint labeling was apparent in pallidum, hypothalamus, substantia nigra, and brainstem. This pattern of distribution corresponds to that of NMDA receptors visualized previously by autoradiography in particular with the antagonist radioligands $^{3}$H-labeled 3-[(±)-2-carboxypiperazine-4-yl]propyl-1-phosphonic acid (CPP), $^{3}$H-labeled AP5, or $^{3}$H-labeled CGP 39653 (refs. 14 and 15; T.M. and H.M., unpublished results). These findings further support the specificity of the photolabeling reaction and underlie the ability of the photolabel to interact with the vast majority of NMDA receptors.

Trophic NMDA Receptors. In cultures of cerebellar granule cells glutamate exerts mainly a trophic action via NMDA receptors (19). It was, therefore, tested whether trophic NMDA receptors can be structurally distinguished from those in adult brain despite their similar ligand affinities. When cerebellar granule cells were photolabeled with 1 nM $^{125}$I-labeled CGP 55802A, specific incorporation of radioactivity was almost exclusively detected in a protein band of 115 kDa (Fig. 5A). An additional faintly photolabeled band of 175 kDa was apparent after overexposure. This photolabeling pattern differs strikingly from that in adult brain, where the 175-kDa band was exclusively labeled. Thus, the NMDA receptors in granule cell cultures are distinguished from those in adult brain by different structural determinants of their ligand-binding site.

Structural Maturation of NMDA Receptors. Like other ligand-gated ion channels (24) the NMDA receptor undergoes changes in its functional properties during development (25, 26). It was therefore tested whether corresponding structural changes could be identified by photolabeling the receptor in early postnatal brain. When brain membranes from P0 and P5 were photolabeled, radioactivity was specifically incorporated into two protein bands with apparent sizes of 175 kDa and 115 kDa. However, in contrast to the receptors described above, both protein bands were labeled (Fig. 5B). Thus, NMDA receptors in early postnatal brain differ in subunit composition from those in adult brain and in cerebellar granule cells.

In NMDA receptors of early postnatal brain (P0 and P5) the incorporation of radioactivity in the 115-kDa band was
slightly higher than in the 175 kDa band (Fig. 5B; ratio of ODs 175/115 = 0.7). However, at P10 photolabeling of brain membranes resulted in a pattern of photoreactive proteins that showed the inverse ratio with a predominance in the incorporation of radioactivity into the 175-kDa band (ratio of ODs 175/115 = 2.7). The preferential photolabeling of the 175-kDa band was even more pronounced at later time points (P21), amounting to an almost exclusive labeling of the 175-kDa band at P30 and in adult brain (Fig. 5B; ratio of ODs = 6.3 at P21, ratio = 8.5 at P30 and adult). This result points to a structural maturation of NMDA receptors, possibly due to genetically controlled subunit substitution.

**Recombinant Receptors.** The 175-kDa photolabeled protein (165 kDa after deglycosylation) corresponds in size to the cloned subunits NR2A and NR2B (calculated polypeptide sizes 165 kDa, refs. 10-12). The 15-kDa photolabeled protein (105 kDa after deglycosylation) corresponds in size to the NR1 subunit (calculated size 105 kDa, ref. 9). It was therefore of interest to determine whether these cloned subunits represented the photoreactive receptor component. When 293 cells were transfected with the cDNA of either of subunits NR1, NR2A, NR2B, or NR2C, no specific photolabeling was observed. However, cotransfection of the cDNAs for the NR1 and NR2A subunits resulted in specific photolabeling of a band of 175 kDa and, with equal intensity, of a rather broad band around 115 kDa (Fig. 6). Deglycosylation reduced these values to 165 kDa and 105 kDa, respectively. Thus, the subunit combination NR1/NR2A, which is known to form gated ion channels (10, 11), gives rise to a photoreactive ligand-binding site. Coexpression of the NR1 subunit with either the NR2B or NR2C subunit likewise results in functional NMDA receptors, as judged electrophysiologically (11, 12). Nevertheless, both subunit combinations failed to result in specific photolabeling, even when the corresponding gels were overexposed. In recent reversible ligand binding with [3H]glutamate on transfected 293 cell membranes, the closely related NMDA receptor antagonist CGP 39653 exhibited high ($K_i < 10 \text{ nM}$) affinity for the NR1/NR2A subunit combination but low ($K_i \gg 500 \text{ nM}$) affinity for all homo- and heteromeric assemblies (D.L. & P.S., unpublished work). This result probably explains the restriction of photolabeling of 125I-labeled CGP 55802A to the NR1/NR2A receptors.

**DISCUSSION**

**Three Types of NMDA Receptors in Situ.** A glycoprotein of 175 kDa was identified that contributes to the agonist/antagonist binding site of NMDA receptors in adult brain. This conclusion is based on several lines of evidence: (i) 125I-labeled CGP 55802A, a photoreactive NMDA antagonist with nanomolar affinity, interacts selectively with a glycoprotein of 175 kDa. (ii) The photoreaction can be inhibited only by ligands of the NMDA-agonist/antagonist-binding site. Their potencies correspond to their IC$_{50}$ values in reversible ligand binding. (iii) The photolabeled site displays stereoselectivity, as shown for the differential interaction of a pharmacologically active and inactive enantiomer of a NMDA-receptor antagonist. (iv) The labeled glycoprotein, which is present in various mammalian species, displays a distribution that corresponds to that of NMDA receptors, previously visualized by various labeled antagonists (14, 15). Thus, the 175-kDa glycoprotein is a structural determinant for the interaction of agonists and antagonists at the NMDA receptor and may, therefore, also be involved in gating of the channel. Previous attempts to photolabel the NMDA receptor largely lacked the necessary selectivity. Photolabeling with [3H]azido-MK801 was shown to be inhibited only by the nonspecific agent phencyclidine (27). [3H]Azidophencyclidine interacted with five different proteins attributed to the phencyclidine/$\alpha$ receptor (28).

Receptor constituents of a size comparable to the 175-kDa protein photolabeled in adult brain were recently cloned and termed NR2A (e1) and NR2B (e2) (10-12). In recombinant receptors, only the subunit combination NR1 and NR2A, but not that of NR1 and either NR2B or NR2C, was susceptible to photolabeling. Thus, the NR2A subunit may represent at

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**Fig. 5.** Photolabeling of NMDA receptors in cerebellar granule (Gran.) cell cultures (A) and in developing brain (B). (A) NMDA receptors in granule cells were photolabeled with 1 nM 125I-labeled CGP 55802A and subjected to SDS/PAGE. The autoradiogram shows total photolabeling (left lane) and nonspecific photolabeling (right lane, in the presence of 2 mM NMDA or 1 mM glutamate). (B) Developmental regulation of the pattern of photolabeled subunits. Brain membranes from different stages of development were photolabeled, as described for A. Equal amounts of protein were applied to each lane. At birth (P0) and P5 the incorporation of radioactivity in the 115-kDa band was slightly higher than in the 175-kDa band. At later time points an inverse ratio of incorporation was seen, pointing to a structural maturation of the NMDA receptor.

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**Fig. 6.** Photolabeling of mammalian cells transfected with cDNAs of NMDA-receptor subunits. Transfection of 293 cells was done with cDNAs coding for the subunits NR1, NR2A, or the subunit combinations NR1 plus NR2A and NR1 plus NR2B. After photolabeling of the cells with 2.5 nM 125I-labeled CGP 55802A with and without 2 mM NMDA, the cells were analyzed by SDS/PAGE with equal amounts of protein used per lane. Photoreactivity was seen only for the subunit combination NR1 plus NR2A. For comparison, photolabeled brain membranes were included in SDS/PAGE (far right lane). T, total labeling; N, nonspecific labeling. The subunit combination NR1 plus NR2C lacked photoreactivity (data not shown).
least one component of the photolabeled protein band of 175 kDa. However, the NMDA receptor in adult brain is not fully represented by the NR1/NR2A subunit combination. In the NR1/NR2A combination both subunits were photolabeled with equal intensity, whereas photolabeling is directed almost exclusively to the 175-kDa band in adult brain. This finding points to the presence of additional components in native NMDA-receptor complexes that convey a photolabeling pattern distinct from that of the NR1/NR2A recombinant receptor. Thus, the subunit combination NR1 and NR2A by itself is insufficient to describe a major population of NMDA receptors in adult brain.

The regional distribution of the mRNA of the NR2A subunit visualized by in situ hybridization (11, 12) partly corresponds to the autoradiographical distribution pattern of the photolabeled protein. Both signals were high, e.g., in cerebral cortex and hippocampus and low, e.g., in hypothalamus. However, striking regional differences in signal intensities were also apparent, e.g., in thalamus where strong photolabeling contrasts with a very low abundance of NR2A mRNA. Thus, in certain brain regions NR1/NR2A subunit combinations other than NR2A, although comparable in size, may exist as photoreactive constituents of NMDA receptors.

A second type of NMDA receptor was demonstrated in cerebellar granule cell cultures, where NMDA receptors mainly mediate the neurotrophic actions of glutamate (19). These trophic receptors display a photolabeling pattern opposite to that of receptors in adult brain in that the protein band of 115 kDa is almost exclusively photolabeled. Thus, trophic NMDA receptors typify a separate type of NMDA receptor. The photolabeled glycoprotein of 115 kDa presumably corresponds to the NR1 subunit. Apart from the correspondence in size, the NR1 subunit was found to be susceptible to photolabeling when coexpressed with the NR2A subunit in 293 cells. Thus, the trophic NMDA receptors may contain both these subunits. However, because the subunit combination NR1/NR2A does not display subunit-selective photoreactivity, the presence of additional components must be postulated to explain the preferential photoreactivity of the 115-kDa protein in the trophic receptors.

A third type of NMDA receptor was identified in early postnatal brain (20). Two protein bands of 175 kDa and 115 kDa were almost equally susceptible to photolabeling in these receptors, in striking contrast to the photolabeling pattern of NMDA receptors in either adult brain or cerebellar granule cell culture. The two components photolabeled in postnatal brain presumably correspond to the NR2A and NR1 subunit, which are both photoreactive when coexpressed in mammalian cells.

Developmental Regulation. The kinetic properties of NMDA-activated channels are developmentally regulated (25, 26), which may reflect structural changes induced by developmentally controlled genetic expression of receptor subunits. We now present evidence for a structural change of NMDA receptors during development. The photolabeling pattern of NMDA receptors at P0 and P5, characterized by both the 175-kDa and 115-kDa bands being photolabeled, is converted to the pattern seen in adult brain where the 175-kDa band is almost exclusively labeled. The switch in receptor structure may be linked to synaptic activity because the labeling pattern of the adult form of the receptor begins to emerge at the time of extensive synaptogenesis (P10). Indeed, an activity-dependent change in NMDA-receptor kinetics around day 10–15 has been described (25, 26). These developmental changes are reminiscent of the developmental regulation of nicotinic acetylcholine receptors at the neuromuscular junction, where a subunit substitution is the basis for the developmental changes in the end-plate current (24).

A similar mechanism may underlie the structural maturation of NMDA receptors.

The photoaffinity ligand of NMDA receptors will be of value not only in dissecting the structural heterogeneity of this class of receptors but also to monitor the regulation of the expression of particular receptor subunits under physiological and pathological conditions.

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