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Q/R editing of the rat GluR5 and GluR6 kainate receptors *in vivo* and *in vitro*: evidence for independent developmental, pathological and cellular regulation

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Abstract

Kainate (KA) is a potent neuroexcitatory agent in several areas of the adult brain, with convulsant and excitotoxic properties that increase as ontogeny proceeds. Besides its depolarizing actions, KA may enhance intracellular accumulation of Ca^{2+} to promote selective neuronal damage. The effects of KA are mediated by specific receptors recently considered to be involved in fast neurotransmission and that can be activated synaptically. KA receptors, e.g. GluR5 and GluR6 have been characterized by molecular cloning. Structure–function relationships indicate that in the MII domain of these KA receptors, a glutamine (Q) or arginine (R) residue determines ion selectivity. The arginine stems from post-transcriptional editing of the GluR5 and GluR6 pre-RNAs, and the unedited and edited versions of GluR6 elicit distinct Ca^{2+} permeability. Using a PCR-based approach, we show that *in vivo*, Q/R editing in the GluR5 and GluR6 mRNAs is modulated during ontogeny and differs substantially in a variety of nervous tissues. GluR5 editing is highest in peripheral nervous tissue, e.g. the dorsal root ganglia, where GluR6 expression is barely detectable. In contrast, GluR6 editing is maximal in forebrain and cerebellar structures where GluR5 editing is lower. Intra-amygdaloid injections of KA provide a model of temporal lobe epilepsy, and we show that following seizures, the extent of GluR5 and GluR6 editing is altered in the hippocampus. However, *in vitro*, high levels of glutamate and potassium-induced depolarizations have no effect on GluR5 and GluR6 Q/R editing. GluR6 editing is rapidly enhanced to maximal levels in primary cultures of cerebellar granule neurons but not in cultured hippocampal pyramidal neurons. Finally, we show that cultured glial cells express partially edited GluR6 mRNAs. Our results indicate that Q/R editing of GluR5 and GluR6 mRNAs is structure-, cell type- and time-dependent, and suggest that editing of these mRNAs is not co-regulated.

Introduction

The amino acid L-glutamate mediates excitatory synaptic transmission in the invertebrate and vertebrate nervous systems (Mayer & Westbrook, 1987). This neurotransmitter plays a primordial role in the development of the brain as well as in synaptic plasticity associated with learning and memory (McDonald & Johnston, 1990; Collingridge & Singer, 1990). Glutamate may also contribute to several neurological diseases associated with selective neuronal cell loss. These include epilepsy or ischaemia, where elevated levels of glutamate may induce excessive activation of glutamatergic receptors (reviewed in Choi, 1992). The effects of glutamate in the central nervous system (CNS) are mediated by distinct receptors, pharmacologically defined by their selective agonists. AMPA- and kainate (KA)-gated receptors (Olney *et al*., 1974) mediate fast synaptic neurotransmission (Monaghan *et al*., 1989; Collingridge & Lester, 1989; reviewed in Lerma *et al*., 1997), and there is recent evidence to suggest that KA receptors can be activated synaptically (Vignes & Collingridge, 1997; Castillo *et al*., 1997). In hippocampal slices, KA induces long-lasting modifications of the synaptic properties of the CA3 pyramidal neurons (Ben-Ari & Gho, 1988). Systemic or intracerebral administration of KA produces a paroxysmal activity which involves preferentially limbic structures and the hippocampus, and leads to neuropathological lesions reminiscent of human temporal lobe epilepsy (Nadler, 1981; Ben-Ari, 1985). Various studies have shown that the convulsant and brain damage syndromes induced by KA are delayed during rat ontogeny (Nitecka *et al*., 1984; Tremblay *et al*., 1984). The late occurrence of high-affinity KA binding sites linked to an immature hippocampal neuronal network have been proposed to contribute to these observations (Berger *et al*., 1984).

Molecular cloning has allowed the initial characterization of members of the glutamate receptor family (Hollmann *et al*., 1989; Gregor *et al*., 1989; Wada *et al*., 1989), and subsequent studies have unravelled some of the properties of families of KA- and AMPA-gated ionotropic glutamate receptors whose members are designated, respectively, GluR5, GluR6, GluR7, KA-1, KA-2 (Bettler *et al*., 1990; Egebjerg *et al*., 1991; Werner *et al*., 1991; Bettler *et al*., 1992; Lomeli *et al*., 1992; Herb *et al*., 1992; Sakimura *et al*., 1992; Sommer *et al*., 1992) and GluR-A–D (or GluR1–4) (Hollmann *et al*., 1989; Keinänen *et al*., 1990; Boulter *et al*., 1990; Nakanishi *et al*., 1990; Bettler *et al*., 1990). These receptor subunits have been grouped on the basis of their sequence and pharmacological properties. They share similar lengths (approximately 900 amino acids) and extensive sequence

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conservation (reviewed in Hollmann & Heinemann, 1994). Structure–function studies in transfected cells have revealed within the second hydrophobic domain (MII) of the GluR variants, the importance of either a glutamine (Q) or arginine (R) residue in determining ion selectivity and rectification properties of channels: while heteromeric or homomeric GluR-A, C and D receptors contain a glutamine in MII, generating strongly inward rectifying receptors permeable to Ca^{2+}, an arginine is found in GluR-B where it elicits low Ca^{2+} permeability and a linear *I–V* relationship (Hume *et al*., 1991; Verdoorn *et al*., 1991; Burnashev *et al*., 1992a). The arginine in the MII domain of GluR-B, as well as in some of the GluR5 and GluR6 subunits, is not encoded by the corresponding genes but results from post-transcriptional nuclear editing of their respective pre-mRNAs (Sommer *et al*., 1991; reviewed in Seeburg, 1996) by double-stranded RNA adenosine deaminases (Bass, 1995; Dabiri *et al*., 1996; Herb *et al*., 1996; Maas *et al*., 1996; Melcher *et al*., 1996a, 1996b; reviewed in Maas *et al*., 1997) that convert adenosine to inosine by enzymatic base modification (Melcher *et al*., 1995; Rueter *et al*., 1995). While the GluR-B mRNA seems to be fully edited, the percentage of GluR5 and GluR6 Q/R editing in adult rat brain structures is comprised between 30–55% and 70–90%, respectively (Sommer *et al*., 1991; Köhler *et al*., 1993; Bernard & Khrestchatisky, 1994; Cha *et al*., 1994; Paschen & Djuricic, 1994, 1995). Q/R editing modulates Ca^{2+} permeability of the GluR6 receptor (Egebjerg & Heinemann, 1993; Köhler *et al*., 1993; Burnashev *et al*., 1995). The importance of editing in MII is further demonstrated by the fact that additional editing in the TMI domain of GluR6 also elicits Ca^{2+} permeability, the extent of which may be modulated by editing in MII (Köhler *et al*., 1993). Finally, the rectification properties of GluR6 seem to be exclusively controlled by Q/R editing and are independent of editing sites of TMI (Ruano *et al*., 1995).

Expression of the mRNAs encoding the KA receptor subunits, GluR5 and GluR6, is broad throughout the CNS and PNS, and decent correlations are found between their combined expression patterns and the distribution of KA binding sites (Bettler *et al*., 1990; Egebjerg *et al*., 1991; Wisden & Seeburg, 1993). This broad distribution of KA receptor subunits in the developing and adult rat CNS, linked to the editing-dependent Ca^{2+} permeability and the different physiological responses, seizure activity and increased neuronal lesions induced by KA in the course of development, has led us to study GluR5 and GluR6 editing in developing nervous tissues and the epileptic hippocampus. We also have assessed whether we could modulate GluR5 and GluR6 Q/R editing in cultured neural cells. Although GluR5 and GluR6 editing for a limited number of brain regions in rat and human have been previously described using various approaches, we present here an extensive comparison of both KA receptors in one species and with one strategy. We show that *in vivo*, the levels of GluR5 and GluR6 Q/R editing differ substantially in adult nervous tissues, and that in all brain structures studied, editing is modulated during ontogeny. GluR5 editing is highest in peripheral nervous tissues, while GluR6 editing is maximal in forebrain and cerebellar structures. In the epileptic rat hippocampus, we show that GluR5 and GluR6 editing is altered. *In vitro*, we show that the extent of editing for both GluR5 and GluR6 differs in cultured hippocampal neurons and primary cultures of cerebellar granule neurons. In the latter, the extent of editing is not modulated by potassium-induced depolarizations, or by high levels of glutamate.

Materials and methods

Animals

Wistar rats ranging from birth (P0) to 21 days old (P21) (three–four animals per age group) or adult (three animals), were killed, and the brain regions of interest and various nervous tissues were rapidly dissected, pooled and processed for RNA extraction. Seizures were induced in rats in the following way: male Wistar rats (180–200 g) were anaesthetized with chloralhydrate and submitted to unilateral injections (0.3 μL) of KA [Sigma, 10 mg/kg, in phosphate buffer (PB), pH 7.4] in the amygdala as previously described (Represa *et al*., 1987). Only those animals showing typical KA-induced limbic motor seizures for at least 2 h upon recovery from anaesthesia were considered for analysis. Animals ($n = 3$ animals at least per time point) were killed 6, 12, 16 and 24 h, 3, 7, 12, 14, 21, 30 days and 6 months after KA treatment. Naive animals and animals injected with 0.3 μL PB at the same stereotaxic coordinates were used as controls ($n = 3$ animals per time point) and were killed at 6, 16, 24 h and 3 days. The hippocampi of the control and epileptic animals were dissected out, pooled and further processed for RNA extraction and cDNA synthesis as described further.

Cell cultures

For hippocampal cell cultures, pregnant Wistar rats were killed on gestational day 18 for collection of the embryos, and primary cultures of hippocampal neurons were prepared as previously described (Ferhat *et al*., 1993, 1997). Cerebellar granule cells were prepared from P7–8 rat pups, as described in Dessi *et al*. (1993), and are devoid of Purkinje cells. Secondary glial cell cultures (2 weeks) were prepared from the dissected hippocampi of P0 rat pups as described previously (Ferhat *et al*., 1994).

RNA extraction and reverse transcription

Using the acid guanidinium thiocyanate–phenol–chloroform extraction method of Chomczynski & Sacchi (1987), total cellular RNA was prepared from different nervous tissues of adult rats (cortex, cerebellum, hippocampus, brain stem, striatum, olfactory bulb, retina, inner ear, thalamus, spinal chord and dorsal root ganglia); from the hippocampi of epileptic rats; from the hippocampi, cerebellum, cortex and olfactory bulb of rats at E18 and postnatal days P0, P4, P7, P10, P14 and P21; from primary cultures of cerebellar granule neurons and hippocampal pyramidal neurons; and from secondary cultures of glial cells. The method was modified to meet the needs of reverse transcriptase-coupled PCR, as described in Ferhat *et al.* (1993). Genomic DNA was eliminated by adding 10 units of Rnase-free FPLC-pure DNase (Pharmacia, Uppsala, Sweden) and incubating for 15 min at 37 °C. Total RNA was quantified on a spectrophotometer, and integrity of the RNA was assessed in a denaturing agarose gel. For all samples, equal amounts of total RNA (1 μg) were reverse transcribed as described previously (Ferhat *et al*., 1993). cDNA input was identical for all PCR reactions. Except when mentioned, the same number of PCR cycles (30) was performed in all experiments. It should be emphasized that although the same RT-PCR procedure was used for all samples, we do not consider the PCRs as quantitative. In the case of the embryonic dorsal root ganglion (DRG) where only minute amounts of tissue were available, RT-PCR was performed directly after tissue disruption at 100 °C. When semiquantitative RT-PCR was performed (assessment of GluR5 increase in the presence of potassium and comparison of GluR6 expression levels in neurons versus glial cells), total RNA (1 μg) from the different cell cultures was spiked with 40 pg of tobacco Nitrate Reductase (NR) RNA transcribed *in vitro*. Following cDNA synthesis and PCR, the NR PCR products were quantified and used as standards, as described in Ferhat *et al*. (1993). In each set of experiments, PCR controls included RT-PCR with no RNA input, mock cDNA synthesis (no RT) and PCR reactions in the absence of cDNA input.

PCR reactions

The primers used in this study are: GluR5/6, 5′-GGTATAACCC(A/C)CACCC(T/A)TGCAACC, an upstream primer common to GluR5 and GluR6, spanning residues WYNPHPCNP on GluR5 (559–567) and GluR6 (558–566) (Egebjerg *et al*., 1991); GluR523, 5′-GAAGGTCATCGTCGAGCCATCTCTG, a downstream primer complementary to the GluR5 mRNA, spanning residues VRDGSTMTF (655–663); GluR62, 5′-TGACTCCATTAAGAAAGCATAATCCGA, a downstream primer complementary to the GluR6 mRNA, spanning residues SDYAFLMES (700–708).

The upstream primer GluR5/6 was end-labelled using [γ-^{33} P] ATP (NEN, Du Pont de Nemours, Les Ulis, France) and T4 polynucleotide kinase (BRL, Gaithersburg, MD, USA) as described in Bernard & Khrestchatisky (1994). PCR reactions [50 µL, 30 cycles, 30 s denaturation at 94 °C, 30 s annealing at 58 °C (GluR5) or 56 °C (GluR6), and 30 s elongation at 72 °C] were performed using 5 µL of the diluted cDNAs.

Gel analysis and autoradiography

Ten microlitres of each PCR reaction was digested with the endonuclease BbvI (New England Biolabs, Beverley, MA, USA). One-third of the digested samples were loaded on 5% polyacrylamide gels. Following electrophoresis, gels were fixed in 10% acetic acid, dried and exposed to Amersham MP films from 2 to 12 h. Autoradiograms were scanned and digitized using Samba software (TITN Alcatel, Grenoble, France). For each measure, the number of pixels corresponds to a mean value of the entire band after it was digitized, so that minor band distortions in the course of electrophoresis have no incidence on the densitometric readings. The least-exposed autoradiograms were used for quantifications so as to avoid film saturation and to ensure measures were realized within the linear range of the films. In experiments with large differences between the levels of edited and unedited RNA (notably GluR6), films were preflashed to avoid non-linearity at the low end of the exposure range. The autoradiograms that were scanned do not always correspond to those used for the figures, which for the sake of illustration may correspond to longer exposures. For the *in vivo* experiments (adult nervous tissues and ontogeny of the hippocampus, cerebellum, cortex and olfactory bulb), two independent batches of cDNAs were assessed. In all cases, at least three different PCR and BbvI digestion reactions were performed from each cDNA sample mentioned in this study. Furthermore, all samples were run at least three times and in some cases up to five times for quantifications. Results were expressed as percentage of edited over the total (edited + unedited). Highly similar results were always obtained, and percentages correspond to the mean of these results. For a given RNA sample that was processed several times (independent RT-PCR, digestion by BbvI and gel analysis), we observed a maximum of 5% difference in the edited percentages. When statistical analysis was performed for significance (ANOVA followed by Fischer) in the *in vitro* experiments, results were averaged from at least three distinct experiments. In the experiments with the rat epileptic tissues (Fig. 3), the hippocampi from at least three animals were pooled at each time point. Two different cDNA batches were prepared, and the digested PCR products from each batch were assessed from four (GluR5) or three (GluR6) independent gels. In order to avoid overload on the figures, standard deviation and statistical significance were plotted only when relevant.

Results

Assessing the extent of GluR5 and GluR6 Q/R editing

The extent of editing in the MII domains of the GluR5 and GluR6 mRNAs was assessed using Reverse Transcription-coupled Polymerase Chain Reaction (RT-PCR) for the amplification of DNA fragments spanning the MII domains. Our approach is only briefly mentioned, as it has already been described in detail elsewhere (Bernard & Khrestchatisky, 1994). Q/R editing of GluR5 and GluR6 suppresses a restriction site for the endonuclease BbvI in the corresponding double-stranded cDNA. RT-PCR primers were chosen such that GluR5 and GluR6 amplification products span the BbvI site of the unedited sequence as well as a ‘constitutive’ BbvI site used in each sample as an internal control for efficient BbvI digestion. The upstream primer is 5′ end-labelled with [γ-^{33}P] ATP. Following BbvI digestion, samples are analysed on a polyacrylamide gel which is further dried and autoradiographed. In a mixed population of edited and unedited mRNAs, RT-PCR yields amplification products of 313 and 451 bp for GluR5 and GluR6, respectively; BbvI digestion products of the following sizes are visible in ethidium bromide-stained gels: 187, 126, 106 and 81 bp for GluR5; and 375, 268, 107 and 76 bp for GluR6 (Bernard & Khrestchatisky, 1994). In either case, of the four digestion products, only two (187 and 106 bp for GluR5, and 375 and 107 bp for GluR6) are end-labelled, as revealed by autoradiograms of the dried gels. In each lane, relative intensities of the two bands are measured by densitometric scannings of the autoradiograms. Ratios of the 187/106 and 375/107 digestion products correspond to those of the edited and unedited GluR5 and GluR6 mRNAs in a given RNA sample.

GluR5 and GluR6 Q/R editing throughout development of different brain structures

We analysed the extent of GluR5 and GluR6 Q/R editing in RNAs extracted from embryonic day 18 (E18) hippocampi, and from the hippocampus, cerebellum, cortex and olfactory bulb in P0, P4, P7, P10, P14, P21 and adult rats. We have also included in our study GluR5 editing in E17 and adult DRG (Fig. 1). Following RT-PCR, BbvI digestion of the PCR products, gel analysis and autoradiography (Fig. 1A,C), densitometric scanning (Fig. 1B,D) reveals substantial differences in the ratios of unedited and edited mRNAs, for GluR5 and GluR6. At E18 in the hippocampus, 90% of GluR5 mRNAs are unedited, and in all structures examined, Q/R editing increases during early postnatal development (Fig. 1A,B). At P0, GluR5 mRNAs are poorly edited (24–28% in the cerebellum, cortex and olfactory bulb, and 16% in the hippocampus). By P4, the extent of editing in all four structures is substantially increased, as 43% (hippocampus, cerebellum and cortex) to 48% (olfactory bulb) of the GluR5 mRNAs are edited at this stage. At P7, a slight decrease in the extent of editing is observed in the hippocampus, cerebellum and olfactory bulb. This drop in the amount of edited GluR5 mRNA is even more obvious at P10 in the cerebellum where the edited mRNAs represent 20% of the total, less than at P0. At P10, in the hippocampus, cortex and olfactory bulb, levels of GluR5 editing are similar to those observed at P4. The levels of edited GluR5 mRNAs are stable in the hippocampus at P14 and P21, and are further increased in the adult where they represent 50% of the total. In the cerebellum, the levels of edited mRNAs increase substantially from P10 to the adult (from 20 to 55%), while the extent of GluR5 editing is stable in the cortex and olfactory bulb from P10 to adult (around 50%). In the DRG, levels of GluR5 editing increase from 11% at E17 to 56% in the adult.

The levels of Q/R editing in GluR6 (Fig. 1C,D) are quite different from those of GluR5 throughout development in the brain structures mentioned above. Similar experiments were performed from the same batches of cDNAs used to assess the extent of GluR5 editing. In the hippocampus at E18, 32% of the GluR6 mRNAs are edited. At P0, the level of editing reaches 75% in the hippocampus and cortex, 90% in the cerebellum and 82% in the olfactory bulb. The extent of GluR6

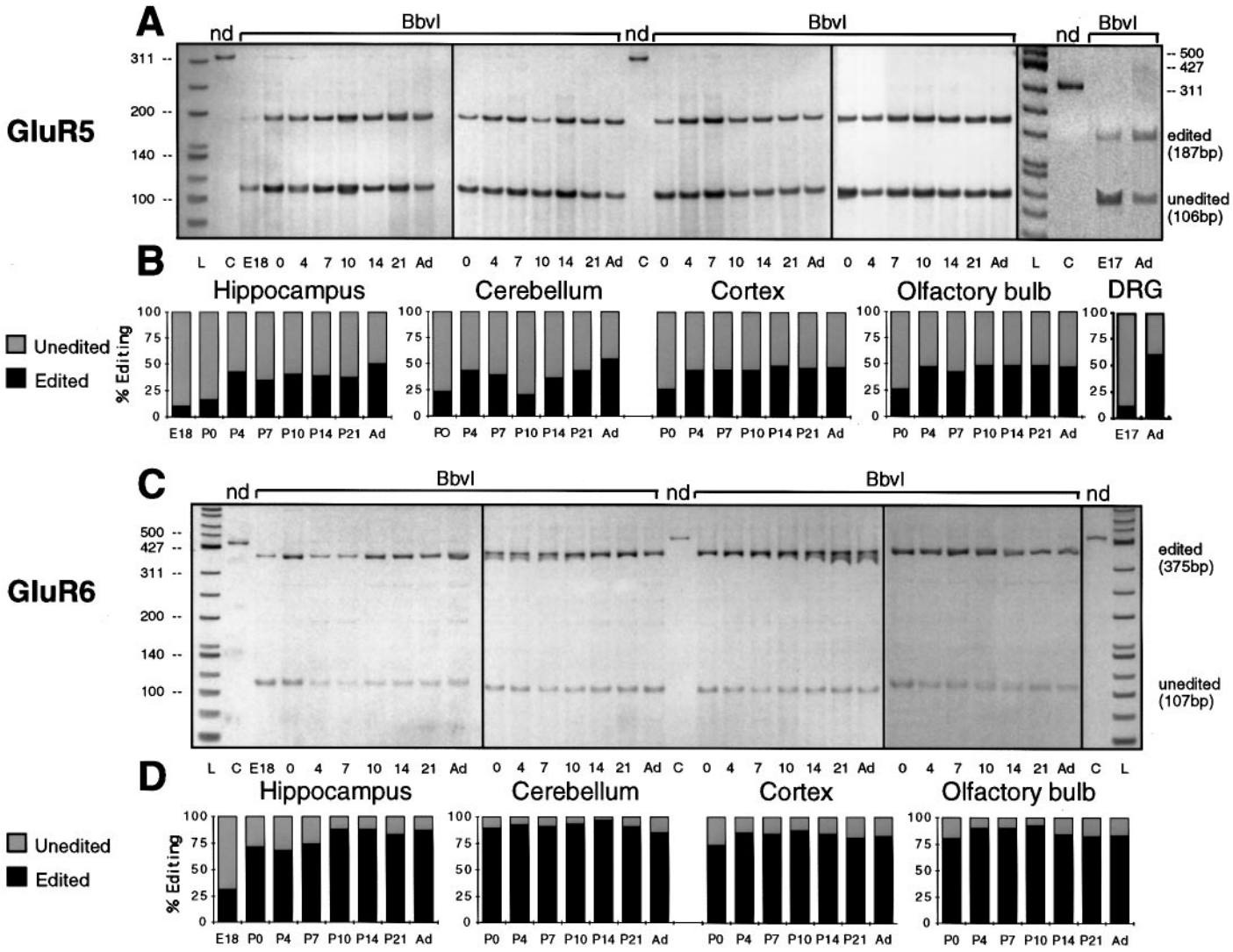

FIG. 1. Q/R editing of GluR5 (A,B) and GluR6 (C,D) during development of the hippocampus, cerebellum, cortex, olfactory bulb and DRG (GluR5). Following RT-PCR, the GluR5- and GluR6-radiolabelled PCR products were digested by the endonuclease BbvI and separated on acrylamide gels, which were further dried and exposed for autoradiography (A,C). Abbreviations: E18, embryonic day 18; 0, 4, 7, 10, 14, 21, postnatal days P0, P4, P7, P10, P14, P21; Ad, adult; (L), labelled Promega ladder; (C and nd), control non-digested PCR products. All other samples were digested by BbvI prior to gel analysis. Autoradiograms were scanned, and the mean percentages of edited GluR5 (B) and GluR6 (D) relative to the total (edited + unedited) were plotted. Data were combined from at least three independent RT-PCR experiments and at least three independent gels

editing is stable in the hippocampus between P0 and P7, and peaks at P10 and P14 (90%). Subsequently, 88% of the GluR6 mRNAs are edited in the adult rat hippocampus. In the cerebellum, we note a slight increase in the extent of editing from P0 to P14, where levels of editing peak and are close to 97%. The percentage of edited GluR6 mRNA decreases slightly at P21 and more so in the adult cerebellum to reach 85%. In the cortex and olfactory bulb, a similar trend is observed: a slight increase in the extent of editing from P0 to P10, where levels of editing peak in both structures to reach 90 and 95%, respectively. Subsequently, a slight decrease in the levels of GluR6 editing from P10 to adult is observed in both structures, where 85% of the GluR6 mRNAs are edited. In the PCR conditions used for the amplification of GluR5 and GluR6 in the tissues mentioned above (30 cycles), we detected no GluR6 in the E17 and adult DRG.

GluR5 and GluR6 Q/R editing in adult nervous tissues

We compared the extent of GluR5 and GluR6 Q/R editing in various central and peripheral nervous tissues of the adult rat (Fig. 2). Total RNA was extracted from cortex, cerebellum, hippocampus, brain stem, striatum, olfactory bulb, retina, inner ear, thalamus, DRG and spinal cord. The highest levels of edited GluR5 mRNAs (Fig. 2A,C) are found in the spinal cord (66%) and retina (62%), where 80% of the GluR6 mRNAs (Fig. 2B,D) are edited. For both GluR5 and GluR6 mRNA species, low levels of Q/R editing are detected in the inner ear (31 and 45% of the total GluR5 and GluR6 mRNAs, respectively). The highest percentage of Q/R editing in adult nervous tissues is found in the GluR6 mRNAs expressed in CNS structures, e.g. the hippocampus (87%), cortex (86%) and cerebellum (83%). In these structures, around 50% of the GluR5 mRNAs are edited. In the brain stem, GluR5 mRNAs are efficiently edited (61%), while editing of the GluR6 mRNAs is substantially lower (63%) than in other areas. Conversely, GluR5 editing is low in the striatum (41%) compared to other nervous tissues, while GluR6 editing in the striatum (84%) is close to the highest levels found in adult tissues. While GluR5 mRNAs are expressed in the DRG and spinal cord at levels that compare with other tissues, GluR6 mRNAs are expressed at low

levels when compared with most other nervous tissues, as three additional PCR cycles are necessary to obtain similar amounts of the PCR product. In the DRG and spinal cord, the low levels of GluR6 mRNA expression are associated with low levels of editing (9 and 46%, respectively) when compared to GluR5 (56 and 66%, respectively).

GluR5 and GluR6 Q/R editing in the rat hippocampus following KA-induced seizures

In order to assess whether neuronal hyperactivity had any effect on the levels of GluR5 and GluR6 editing, we analysed the RNAs extracted from the hippocampi of rats submitted to unilateral injections of KA in the amygdala as previously described (Represa *et al*., 1987).

Six hours after the onset of seizures, in the adult rat hippocampus we observe a significant increase in GluR5 editing, from 50–55% in the different control animals to 69% (Fig. 3A,B). This difference with the control animals is still prominent at 16 h (65% compared to the 51% of the saline-injected control). The ratios of the edited version decrease at 24 h and 3 days following KA (58 and 60%, respectively), while the saline-injected controls remain stable (55 and 53%, respectively). A second wave of modulation of the extent of GluR5 editing seems to occur in the days and weeks following the initial seizures. Indeed, 7 days after KA, high levels of GluR5 editing (71%) are observed in the hippocampus, followed by a slow decrease at 14 days (68%), 21 days (65%), 30 days (62%) and 6 months (60%), where GluR5 editing is higher than in the hippocampi of non-epileptic control animals (50–55%).

In the early time points following seizures (6 h to 3 days), modulations in GluR6 editing (comprised between 86 and 92%) are not comparable with those observed for GluR5 and are not statistically significant (Fig. 3C,D). In contrast with the second wave of GluR5 increase observed 3 days and beyond for the GluR5 subunit, at 3 and 7 days we observe a significant decrease in the extent of GluR6 editing which reaches ratios (80%) that have not been observed in the non-epileptic adult hippocampus. Six months after the onset of seizures, the extent of GluR6 editing is identical to control levels (87%).

GluR5 and GluR6 Q/R editing in cerebellar granule neurons with high glutamate

In order to study *in vitro* whether neuronal activity could modulate Q/R editing of GluR5 and GluR6, primary cultures of cerebellar granule neurons were grown in different conditions. Cerebellar granule neurons were dissected from rat pup cerebella at P7–8. The extent of GluR5 and GluR6 mRNA editing was assessed in the cell suspension [time point (0 h)], and after 6, 24, 48 and 72 h in culture. We observe a significant change in the percentage of edited GluR5 (Fig. 4A,B) after 6 h in culture when compared to the cell suspension [time point (0)]; the percentage of edited GluR5 mRNAs shifts from 35 to 20%. At 24 and 48 h, GluR5 editing levels increase significantly, as editing levels reach 30 and 35% at 24 and 48 h, respectively, after plating and are stable up to 11 days (not shown) *in vitro*. A decrease is observed subsequently, and at 25 days *in vitro*, the edited GluR5 mRNAs represent 20% of the total (not shown). In the cerebellar cultures, at all stages, the levels of the unedited GluR5 variant are higher than those of the edited variant. In these cultures we have not observed a down-regulation in the expression of GluR5 mRNA as observed *in vivo* in mature cerebellar granule neurons (Bettler *et al*., 1990; Wisden & Seeburg, 1993). In the same RNA populations, in the cell suspension [time point (0 h)] and 6 h after plating, the levels of GluR6 editing are similar to those observed *in vivo* in the

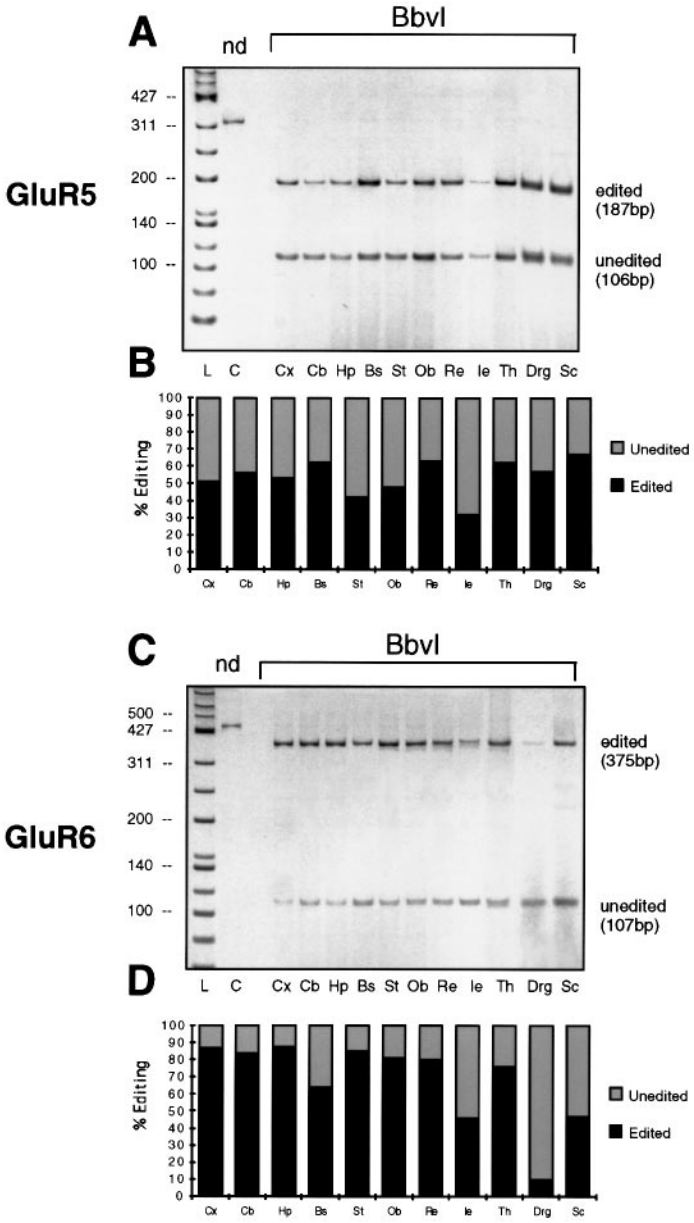

FIG. 2. Q/R editing of GluR5 (A,B) and GluR6 (C,D) in various nervous tissues from the adult rat. Abbreviations: Cx, cortex; Cb, cerebellum; Hp, hippocampus; Bs, brain stem; St, striatum; Ob, olfactory bulb; Re, retina; Ie, inner ear; Th, thalamus; Drg dorsal root ganglia; Sc, spinal cord; (L), labelled Promega ladder; (C and nd), control non-digested PCR products. All other samples were digested by BbvI prior to gel analysis. Three additional PCR cycles were necessary for GluR6 amplification from the DRG and spinal cord cDNAs. Autoradiograms were scanned, and the mean percentages of edited GluR5 (B) and GluR6 (D) relative to the total (edited + unedited) were plotted. Data were combined from at least three independent RT-PCR experiments and at least three independent gels.

cerebellum of developing rats at P4 and P7 (90 and 86%, respectively, Fig. 4C,D). Thereafter, GluR6 editing increases significantly to 100% at 24 h after plating, and subsequently up to 25 days *in vitro* (not shown), the GluR6 Q/R site is exclusively edited (Fig. 4D).

Next, in cerebellar granule neurons prepared from P7–8 rat pups, we assessed whether elevated glutamate levels could modulate the extent of GluR5 and GluR6 editing (Fig. 4E–G). Two days after plating, cells were grown for 1, 2, 4, 8, 24, 48 and 72 h in the presence of 100 μM glutamate. In freshly plated cerebellar granule

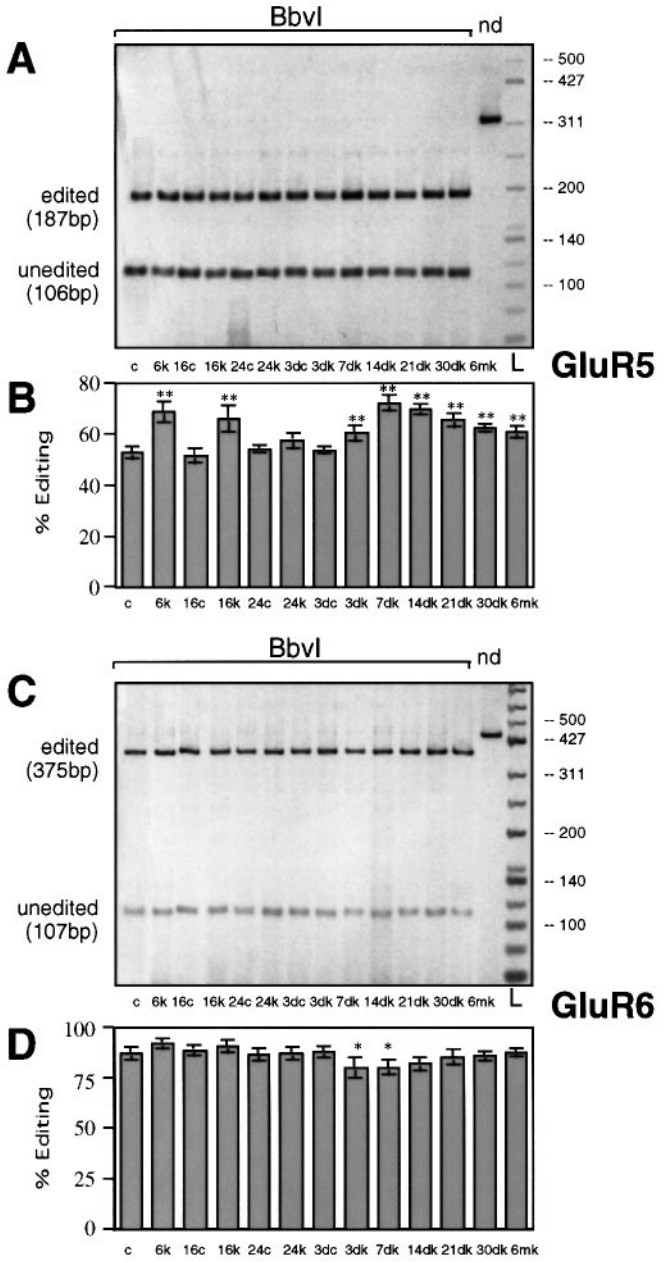

FIG. 3. Q/R editing of GluR5 (A,B) and GluR6 (C,D) in the hippocampi of epileptic rats at various times after intra-amygdalar KA injection. Abbreviations: (c), control hippocampus (naive rats); (6k), 6 h post-KA; (16c), 16 h saline-injected control; (16k), 16 h post-KA; (24c), 24 h saline-injected control; (24k), 24 h post-KA; (3dc), 3 days saline-injected control; (3dk), 3 days post-KA; (7dk), 7 days post-KA; (14dk), 14 days post-KA; (21dk), 21 days post-KA; (30dk), 30 days post-KA; (6mk) 6 months post-KA; (L), labelled Promega ladder; (nd), control non-digested PCR products. All other samples were digested by BbvI prior to gel analysis. Autoradiograms were scanned, and the mean percentages of edited GluR5 (B) and GluR6 (D) relative to the total (edited + unedited) were plotted ± SD. The hippocampi from at least three rats were pooled at each time point. Data were combined from two independent RT-PCR experiments, and from four (GluR5) and three (GluR6) independent gels. Significantly different from the control (c) values: (*) $P < 0.05$; (**) $P < 0.001$.

cells, this concentration of glutamate does not affect cell viability. The extent of GluR5 and GluR6 editing was compared to two control cultures [C, corresponding to time point (0) before the addition of glutamate, and 48C corresponding to 48 h in the absence of glutamate]. In this set of experiments, GluR5 (Fig. 4E,F) and GluR6 (Fig. 4G) RNA editing levels remain identical to both controls.

GluR5 and GluR6 Q/R editing in cultured cerebellar granule cells with high levels of potassium

Next, we assessed whether potassium-induced depolarization could modulate editing of GluR5 and GluR6 (Fig. 5). Cerebellar granule neurons were prepared from P7–8 rat pups. It is known that in established cultures, the transition from high (25–30 mM K^+) to low potassium (5 mM K^+) induces neuronal death (D'Mello *et al*., 1993; Yan *et al*., 1994). It was thus not possible in the course of the culture to first decrease K^+ to observe the subsequent effects of increased K^+ on GluR editing. In order to circumvent this problem, cerebellar granule neurons were plated directly in low K^+. However, because low K^+ is detrimental to cultured neurons after a few days (Gallo *et al*., 1987), we started to evaluate the effects of high potassium (25 mM K^+) on GluR editing rapidly (6 h) after neurons were plated, hence, at an earlier time point after cell dissociation than for the experiments in Fig. 4. At 6 h, in 5 and 25 mM potassium, the levels of GluR6 and GluR5 editing were 82–81% and 18–17%, respectively (Fig. 5A,B), in agreement with other granule cell cultures evaluated early after plating (see Fig. 4). The 48 h cultures showed significantly increased editing for both GluR6 and GluR5 with levels of 95–96% and 33%, respectively (Fig. 5A,B), similar to the levels observed in other experiments (Fig. 4). However, this increase was identical in 5 or 25 mM potassium. Using semiquantitative RT-PCR (gel and NR standard not shown, but the increase is obvious on the GluR5 gel), we observe that the high potassium levels significantly increase the total amounts of GluR5 mRNAs as early as 6 h (300% increase), and that this increase is enhanced at 48 h (500% increase), while GluR6 mRNA expression is not changed (Fig. 5C).

GluR5 and GluR6 Q/R editing in cultured hippocampal neurons

In the rat hippocampus, a substantial shift is observed *in vivo* in the extent of GluR5 and GluR6 Q/R editing between E18 and P4 (Fig. 2). In order to assess whether this shift occurred in hippocampal neurons *in vitro*, as for the cerebellar granule cells, we plated hippocampal pyramidal neurons dissociated from the hippocampi of E18 rat embryos. The extent of GluR5 mRNA editing was 13% in the cell suspension (not shown), close to the 10% observed in hippocampi from E18 rat embryos (Fig. 2), and was assessed in the cultured neurons after 1, 2, 4 and 7 days *in vitro* (Fig. 6A,B). After 24 h in culture, GluR5 editing levels are 20%, and subsequently, we observe a significant decrease to 12% at 7 days in culture. This percentage is substantially lower than that observed at P4 in the developing hippocampus (42%).

Editing of the GluR6 mRNAs increases significantly from 35% in the E18 hippocampal cell suspension, in agreement with the 31% measured in samples of E18 hippocampi (Fig. 2), to 44–43% at days 1 and 2, and to 57–58% after 4 and 7 days *in vitro* (Fig. 6C,D). Again, editing levels of GluR6 are lower than those observed at P4 in the hippocampi of live animals (68%). In contrast with the cerebellar granule cell cultures, a substantial percentage of unedited GluR6 is present in the primary cultures of hippocampal pyramidal neurons, even after 7 days in culture.

GluR6 Q/R editing in cultured glial cells

We next assessed whether GluR editing occurred in secondary cultures of glial cells (Fig. 7). RT-PCR performed in identical conditions from 1 μg total RNA extracted from cultured glial cells and control cultured

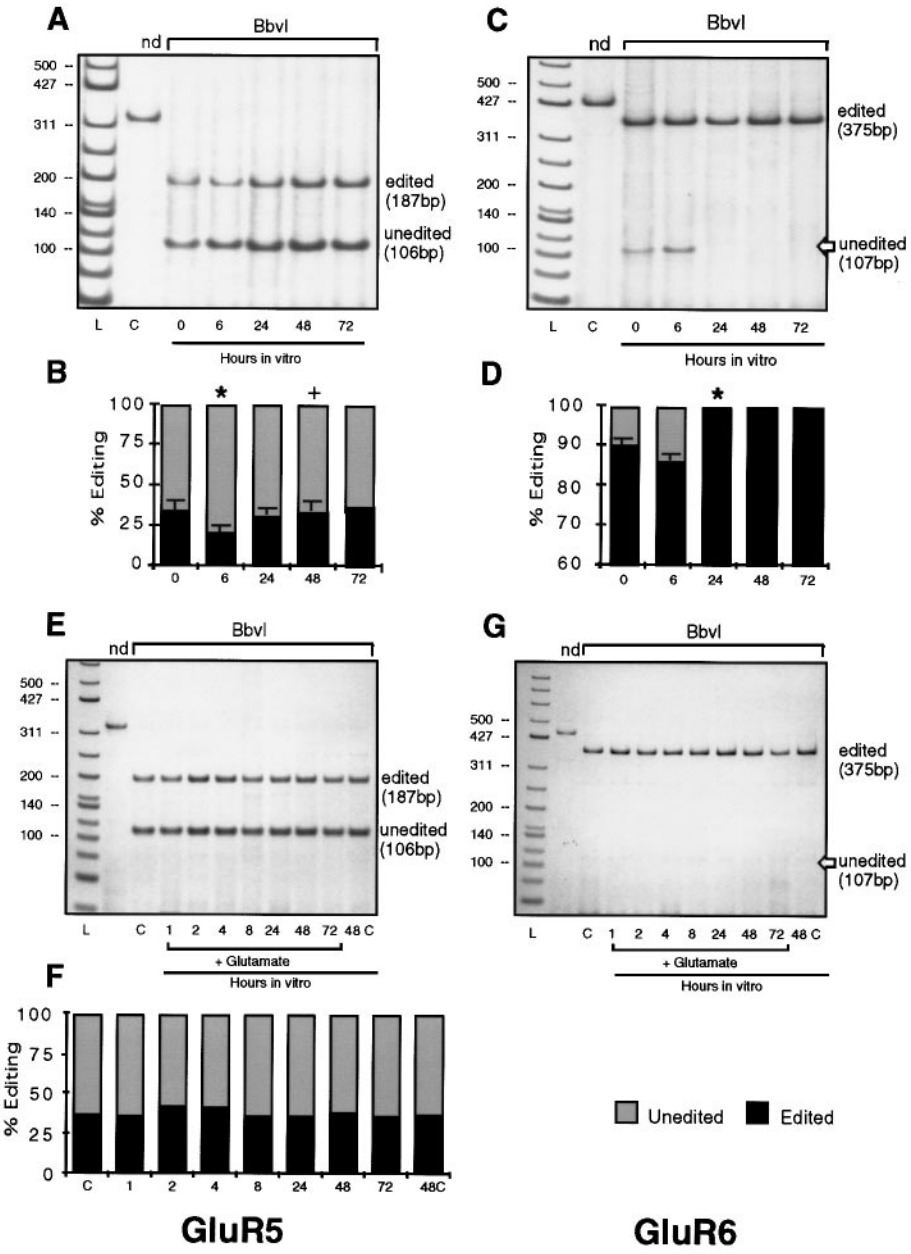

FIG. 4. Q/R editing of GluR5 (A,B) and GluR6 (C,D) in freshly dissociated (time point 0, from P7–8 rat pups) cerebellar cells, and in cultured cerebellar granule neurons for 6, 24, 48 and 72 h. Autoradiograms were scanned (B,D), and the mean percentages of edited GluR5 and GluR6 relative to the total (edited + unedited) were plotted. Data were combined from three cultures. GluR5 editing decreased significantly at 6 h compared to time point (0) [$P < 0.05$, (*)]. At 48 h, GluR5 editing was increased significantly [$P < 0.05$, (+)]. Twenty-four hours following dissociation (time point 0), GluR6 editing was significantly increased [$P < 0.001$, (*)] and reached maximal levels (100%). (E–G) Q/R editing of GluR5 (E,F) and GluR6 (G) in cultured cerebellar granule neurons (2 days *in vitro* from P7–8 rat pups) in the absence (C, 48C) and presence of 100 μM glutamate for 1, 2, 4, 8, 24, 48 and 72 h. Autoradiograms were scanned, and the mean percentages of edited GluR5 (F) relative to the total (edited + unedited) were plotted. Data were combined from three cultures. In the cultured cerebellar granule neurons, after 2 days *in vitro*, GluR6 mRNAs were fully edited. Glutamate had no effect on GluR5 and GluR6 Q/R editing. Abbreviations: (C), control before glutamate; (48C), control culture 48 h in the absence of glutamate; (L), labelled Promega ladder; (nd), control non-digested PCR products. All other samples were digested by BbvI prior to gel analysis.

hippocampal neurons reveals that GluR5 mRNAs are not detected in cultured glial cells (not shown). In contrast, GluR6 mRNAs are expressed in glial cells, and semiquantitative PCR (NR standard not shown) reveals that levels are significantly lower (sevenfold) than in cultured hippocampal neurons (Fig. 7A,B). Assessment of the extent of editing shows that 70% of the GluR6 mRNAs are edited in the cultured glial cells (Fig. 7C).

Discussion

The GluR5 and GluR6 mRNAs are expressed in a variety of nervous tissues during ontogeny, and in the adult central and peripheral nervous systems

Our RT-PCR results confirm that the GluR5 mRNAs are expressed in structures that undergo neurogenesis postnatally, e.g. the olfactory bulb, hippocampus (dentate gyrus) and cerebellum (granule neurons) (Bettler *et al*., 1990). In the latter structure, it has been shown that GluR5 mRNA expression is elevated but transient in the granule cells, and that beyond P12, expression decreases (Bettler *et al*., 1990) and is low in the adult (Bettler *et al*., 1990; Wisden & Seeburg, 1993), while high levels of expression are observed throughout postnatal development in the Purkinje cells. This suggests that up to P10–12, the PCR products we obtain originate from both granule neurons and Purkinje cells, while in the adult cerebellum, it is mostly the Purkinje cell GluR5 mRNA that is amplified. We find GluR5 mRNA in the retina, where it has been localized in the inner nuclear layer (Hughes *et al*., 1992; Müller *et al*., 1992). In agreement with previous results (Bettler *et al*., 1990; Wisden & Seeburg, 1993), we also find GluR5 in the hippocampus, thalamus, striatum, brain stem and spinal cord. In the embryonic and adult rats, we find that the DRG exhibit high levels of GluR5 expression. KA receptors have been described in the C-fibres of the dorsal root ganglia (Agrawal & Evans, 1986). Furthermore, cultured DRG neurons express KA receptors (Huettner, 1990), and it has been suggested that GluR5

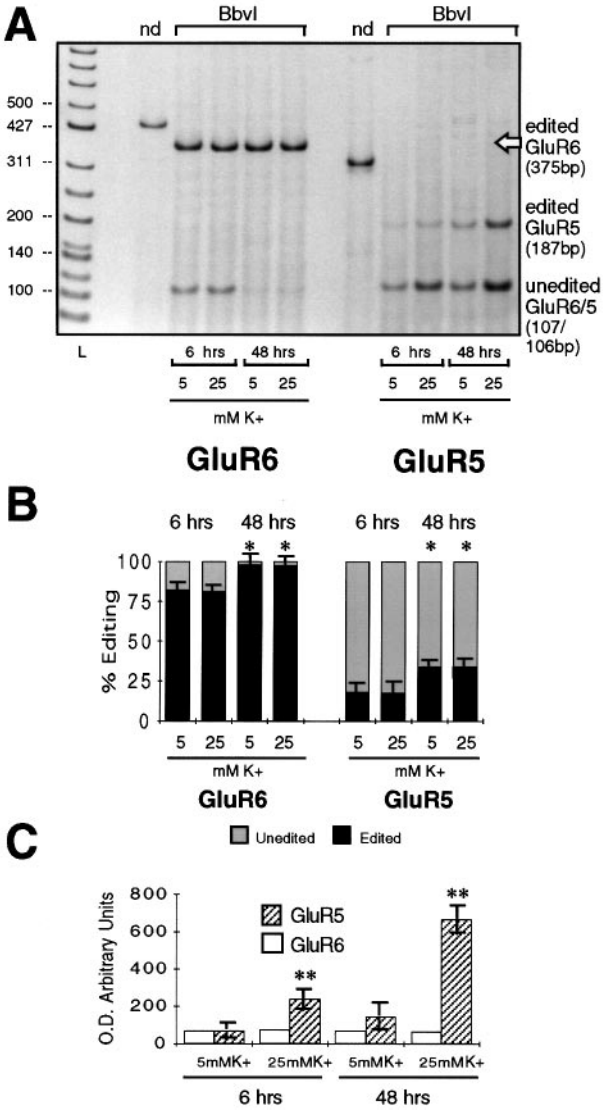

FIG. 5. Q/R editing of GluR5 and GluR6 (A,B) in cultured cerebellar granule neurons (6 h *in vitro*, from P7–8 rat pups) in 5 and 25 mM potassium (K^+) for 6 and 48 h. Abbreviations: (L), labelled Promega ladder; (nd), control non-digested PCR products. All other samples were digested by BbvI prior to gel analysis. Autoradiograms were scanned (B), and the mean percentages of edited GluR5 and GluR6 relative to the total (edited + unedited) were plotted. Data were combined from three cultures. In the freshly dissociated cerebellar granule neurons (6 h *in vitro*), GluR6 mRNAs were not fully edited. After 48 h in culture, they were nearly fully edited and values were significantly different compared to 6 h ($P < 0.05$). Editing of GluR5 also increased significantly at 48 h in culture compared to 6 h ($P < 0.05$). Increased potassium had no effect on GluR5 and GluR6 editing, but significantly increased the global levels of GluR5 mRNA (C) at 6 and 48 h ($P < 0.001$).

constitutes a major component of the glutamate receptor or part of a heteromeric receptor complex in DRG neurons of primary afferent C-fibres (Sommer *et al*., 1992).

Similarly, GluR6 PCR products were obtained from structures shown previously to express GluR6 mRNAs, e.g. the olfactory lobe, hippocampus, thalamus, striatum, cortex, several brain stem nuclei, and retina (ganglion cell layer) (Egebjerg *et al*., 1991; Wisden & Seeburg, 1993). GluR6 PCR products obtained from the cerebellum presumably originate from the granule neurons, which have been shown by these authors to express high levels of the GluR6 mRNA. We also detected GluR6 mRNA in the spinal cord and adult rat DRG, but at low levels, as three additional PCR cycles were necessary to obtain amounts of the PCR product comparable with the other tissues.

Also in this study we used primary cultures of neuronal cells and secondary cultures of glial cells. Primary cultures from developing cerebellum and hippocampus are distinct but rather homogeneous neuronal populations. The primary cultures of cerebellar neurons prepared from the cerebella of P7–8 rat pups are enriched in granule neurons, and it is likely that the amplification products we obtained in these cultures readily originate from the granule neurons for a number of reasons. First, Purkinje cells arise early in the development, and have formed extensive processes and synaptic connections by the time of dissociation; they are irreversibly damaged upon tissue dissociation and we have not been able to detect any Purkinje cells in our cultures. Second, GluR5 (early postnatally), and GluR6 mRNAs are expressed in granule neurons *in vivo* (Bettler *et al*., 1990; Egebjerg *et al*., 1991). Third, unedited and edited versions of GluR5 transcripts have both been detected *in vitro* in single cerebellar granule neurons using single cell RT-PCR (Pemberton *et al*., 1998). At E18, primary cultures from the hippocampus are enriched in pyramidal neurons (Ferhat *et al*., 1993, 1997), and it is most likely that the PCR products we obtained originate from this neuronal cell type. Indeed, as mentioned above, both GluR5 and GluR6 mRNAs are expressed (low levels for GluR5) in the pyramidal cell layer of the rat hippocampus (Bettler *et al*., 1990; Egebjerg *et al*., 1991; Wisden & Seeburg, 1993), and have been characterized in CA1 pyramidal neurons in hippocampal slices (Mackler & Eberwine, 1993). Finally, in cultured glial cells, we find GluR6 mRNA expression, but not GluR5, and at levels that are lower than in cultured hippocampal neurons. It is established that glial cells express functional receptors for glutamate and KA (Bowman & Kimelberg, 1984; Sontheimer *et al*., 1988; Burnashev *et al*., 1992b; Patneau *et al*., 1994).

The extent of GluR5 and GluR6 Q/R editing is differentially regulated during ontogeny, and in the adult central and peripheral nervous systems

The RT-PCR-based method we used (Bernard & Khrestchatisky, 1994) to assess the extent of Q/R editing in the mRNAs encoding GluR5 and GluR6 is simple, reliable and highly reproducible. GluR5 and GluR6 PCR primers were chosen such that they span the MII and TMIII domains, encoded by distinct exons, separated by several kilobases of intronic sequence (Sommer *et al*., 1991). This excludes the possibility that the PCR-amplified products that are analysed derive from contaminating genomic DNA. The amplified products do not allow us to discriminate between the various GluR5 and GluR6 splice variants that have been described in the CNS (Bettler *et al*., 1990; Sommer *et al*., 1992; Gregor *et al*., 1993), but cloned GluR cDNAs show that the amino acid substitution in the Q/R site seems to occur independently of either C- or N-terminal variability (Sommer *et al*., 1992).

Levels of editing measured by RT-PCR in a few structures are in agreement with the results obtained by others following the sequencing of multiple cloned PCR products overlapping the MII domain, or following plaque lifts and the hybridization of oligonucleotides that discriminate the edited and unedited GluR subunits (Sommer *et al*., 1991; Köhler *et al*., 1993). Variants of the approach we described (Bernard & Khrestchatisky, 1994) have been used by various authors (Cha *et al*., 1994; Paschen *et al*., 1994a, 1994b), including at the single cell level (Ruano *et al*., 1995). In some adult rat brain structures, we find results similar to those of Paschen & Djuricic (1995) for GluR6 Q/R editing. The differences between our results and those published by Paschen *et al*. (1995) in adult rat brain for GluR5 editing

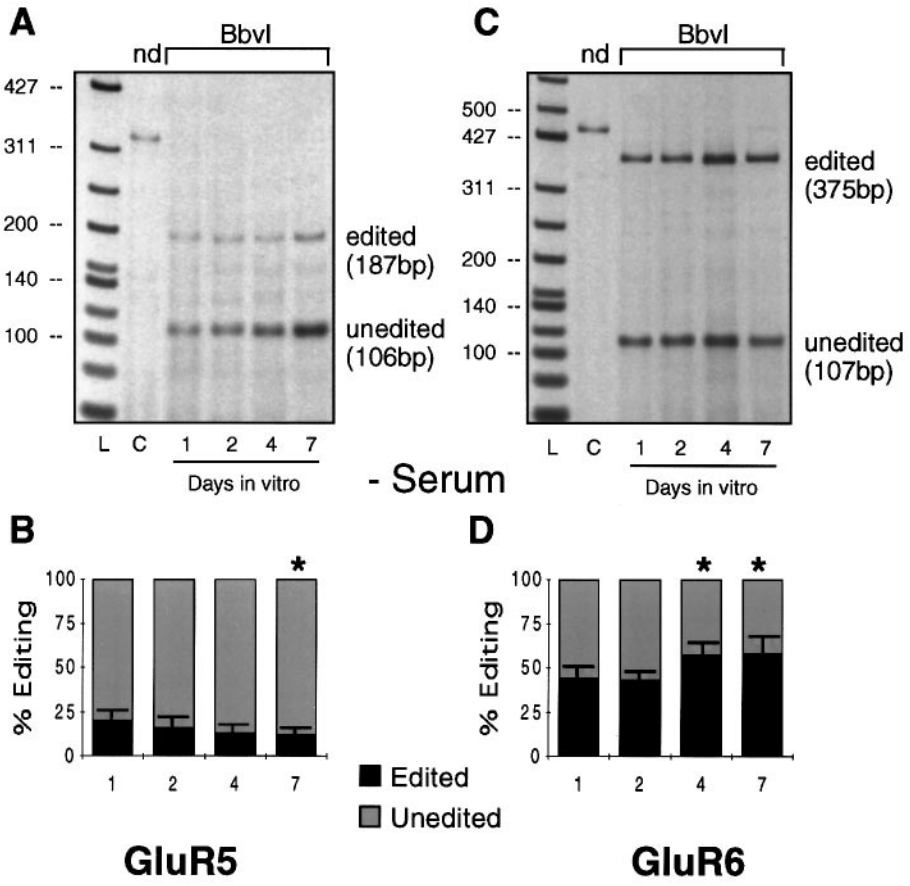

FIG. 6. Q/R editing of GluR5 (A,B) and GluR6 (C,D) in hippocampal neurons after 1, 2, 4 and 7 days *in vitro*. Abbreviations: (L): labelled Promega ladder; (C and nd): control non-digested PCR products. All other samples were digested by BbvI prior to gel analysis. Autoradiograms were scanned (B,D), and the mean percentages of edited GluR5 and GluR6 relative to the total (edited + unedited) were plotted. Data were combined from three cultures. GluR5 editing decreased significantly ($P < 0.05$) between 1 and 7 days *in vitro*. GluR6 editing was slightly but significantly ($P < 0.05$) increased at 4 and 7 days in culture when compared to 1 and 2 days *in vitro*.

may stem from the differences in strategies that were adopted, or from age or strain differences between rats.

In the hippocampus, at E18, GluR5 mRNAs are largely unedited, and the percentage of GluR5 Q/R editing increases in the course of hippocampal development. A similar trend is observed for the cerebellum, cortex and olfactory bulb starting at P0. In some tissues, the switch from unedited to edited is not linear during ontogeny, as a transient but marked decrease of the percentage of edited GluR5 is observed between P7 and P10. This decrease, most obvious in the hippocampus and cerebellum, may be due to the delayed appearance and maturation of specific cell populations, e.g. the granule cells in the dentate gyrus or cerebellum, which presumably initially express unedited GluR5 mRNAs.

At E18, in the hippocampus, the extent of GluR6 Q/R editing is higher than that of GluR5. The same holds true throughout the development of the hippocampus and other structures, where the edited version of GluR6 predominates over the unedited version as early as P0. In contrast with GluR5, no decrease in the extent of GluR6 editing is observed between P7 and P10 in the hippocampus and cerebellum, suggesting that editing of the GluR6 RNA occurs rapidly during maturation of their respective granule neurons.

In any case, we show that the developmental expression of GluR5 and GluR6 is associated with an increase in Q/R editing in agreement with our previous results comparing Q/R editing in the entire embryonic and adult rat brain, and with those of Paschen *et al*. (1995) showing increased GluR5 editing during ontogeny. Our results indicate that the GluR5 mRNAs involved in the early stages of development in areas of neuronal differentiation and synapse formation (Bettler *et al*., 1990) are essentially unedited. In contrast, it seems as if GluR6 mRNAs, when expressed, are rapidly and extensively edited in the Q/R site, suggesting that Ca^{2+} permeability of GluR6 KA receptors may be altered during ontogeny (Köhler *et al*., 1993; Egebjerg & Heinemann, 1993; Burnashev *et al*., 1995). While KA induces intense seizure activity in rat pups, brain damage is not evidenced until the third postnatal week and increases thereafter (Nitecka *et al*., 1984; Tremblay *et al*., 1984). Considering the important contribution of GluR6 in KA-induced epileptogenesis and neuronal damage (Mulle *et al*., 1998), it is tempting to speculate on a link between increased GluR editing and increased sensitivity to KA in the course of development.

In the different adult rat nervous tissues we studied, we show obvious differences in the extent of GluR5 and GluR6 Q/R editing. In all the nervous tissues, except the spinal chord and DRG, the extent of Q/R editing is substantially higher for GluR6 (80–90% on average, in CNS structures, e.g. hippocampus, cortex, striatum and cerebellum) than for GluR5 mRNAs (50% on average). However, in most tissues, the extent of GluR5 and GluR6 editing is not parallel, and in some cases, e.g. spinal cord and DRG, even opposite, i.e. high levels of GluR5 editing (66 and 56%, respectively) are associated with very low levels of GluR6 editing (46 and 9%, respectively) relative to other structures. In the DRG and spinal cord, the low levels of GluR6 editing are associated with low levels of GluR6 expression. In the retina, GluR5 and GluR6 editing presumably takes place in different cell types, as the GluR5 mRNA is mainly expressed in the inner nuclear layer (Hughes *et al*., 1992; Müller *et al*., 1992), while GluR6 mRNA is expressed in the ganglion cell layer (Egebjerg *et al*., 1991).

In general, it appears that GluR6 mRNAs are more efficiently edited at the Q/R site in forebrain and cerebellar structures than in the brain stem and spinal cord, or peripheral nervous tissues, e.g.

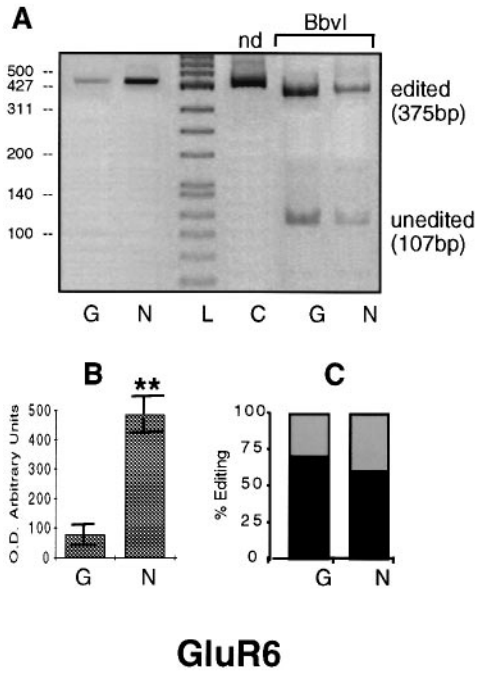

FIG. 7. Q/R editing of GluR6 (A,C) in secondary cultures of glial cells. Abbreviations: (G), glial cells; (N), hippocampal neurons *in vitro*; (L), labelled Promega ladder; (C and nd), control non-digested PCR products. On the left part of the gel, semiquantitative PCR showing low levels of GluR6 mRNA in cultured glial cells when compared to hippocampal neurons. (B) Quantification showing significant ($P < 0.001$) differences in GluR6 expression in glial cells compared to neuronal cells. On the right part of the gel, evaluation of GluR6 editing in cultured glial cells compared with cultured hippocampal neurons. (C) The mean percentages of edited GluR6 relative to the total (edited + unedited) in the glial and hippocampal cell cultures.

DRGs, inner ear or retina. With the exception of the inner ear, the opposite conclusion seems to prevail for Q/R editing of GluR5.

GluR5 and GluR6 Q/R editing are independently altered following KA-induced seizures

At 6 and 24 h following KA treatment, GluR5 Q/R editing levels are increased in the hippocampus. These changes precede neuronal lesions, as the first signs of neuronal death are observed 12 h following treatment (Pollard *et al*., 1994). They also precede synaptic reorganization, as the first signs of mossy fibre sprouting are detected around 12 days after KA (Represa *et al*., 1987). Therefore, it is likely that the early changes in GluR5 editing are a direct consequence of the paroxysmal activity occurring during status epilepticus, and it is apparent from our *in vitro* data with the cerebellar granule neurons that the extent of editing can be rapidly modulated.

We also observe a delayed increase of GluR5 editing in the days and weeks that follow the onset of seizures that seems to be associated with a slight decrease in GluR6 Q/R editing. Interestingly, similar observations were reported (Paschen *et al*., 1996) following cerebral ischaemia, where GluR5 editing was significantly increased in the striatum, a structure vulnerable to ischaemia, while GluR6 editing was reduced. The modulations in editing could be moderate and homogeneous in a large number of cells, or could be more dramatic in a restricted number of cells or given structure. Indeed, in the hippocampus, KA-induced seizures are associated with rapid (few days) cell death of the CA3 pyramidal neurons ipsilateral to the site of KA injection, with sprouting of the mossy fibres (few weeks, Represa *et al*., 1993, 1994) whose terminals are enriched in KA binding sites (Represa *et al*., 1987), and with extensive gliosis (reviewed in Represa *et al*., 1995). Neosynaptogenesis has also been evidenced in the epileptic hippocampus with increased density of KA binding sites (Represa *et al*., 1987). If the GluR5 and GluR6 subunits contribute to novel KA binding sites, it is likely that their expression will be associated with modulation in the extent of editing. It is also possible that the important glial reaction (glial hypertrophy and proliferation) in the degenerating CA3 area and the hilus, which is evidenced only 3 days after KA administration (Represa *et al*., 1995), contributes to changes in GluR6 editing. Indeed, we show (present study) in cultured glial cells that GluR6 editing is lower than that observed in most tissues *in vivo*. Interestingly, Patneau *et al*. (1994) show that the GluR6 mRNA is expressed abundantly in glial cells of the O-2A lineage, while Represa *et al*. (1993) have shown that reactive glial cells in the hippocampus of the KA-treated rats acquire immunoreactivity to antibodies that label the O-2A lineage *in vitro*.

In cultured cerebellar granule neurons, the Q/R site of GluR6 is fully edited, while GluR5 and GluR6 Q/R editing is not affected by glutamate or high potassium

Epilepsy is associated with neuronal hyperactivity and increased glutamate levels. Neuronal activity, glutamate and its agonists, and potassium-induced depolarizations in acute slices or cultured neurons modulate to some extent the expression of specific sets of genes, including those encoding specific subunits of glutamate receptors (Pollard *et al*., 1993; Audinat *et al*., 1994; Bessho *et al*., 1994; Resink *et al*., 1995; Gerfin-Moser *et al*., 1995; Rafiki *et al*., 1998). In this context, we show *in vitro* that high potassium levels rapidly induced GluR5 (but not GluR6) mRNA levels in cultured cerebellar neurons. However, in these neurons, we found that neither increased glutamate levels nor potassium-induced depolarizations had any effect on the extent of GluR5 or GluR6 Q/R editing in the short term. Our results suggest, at least in the cerebellar granule neurons, that modulation of Q/R editing is not activity dependent. The possibility remains that the specific activation of KA receptors themselves elicits signal transduction pathways that modulate Q/R editing. We show rather rapid modulation of GluR5 and GluR6 Q/R editing in cultured neurons, most notably in the cultured cerebellar granule cells. This may be due to the editing process itself or to important changes in the neural populations at stake: it is known that cell dissociation and plating kills preferentially some cell types, e.g. the Purkinje cells which are absent from our cultures and which *in vivo* contribute in part to the extent of GluR5 editing. In the cultured cerebellar granule neurons, we show a very rapid increase and a maximal level of GluR6 editing, similar to that observed for the fully edited GluR-B subunit of the AMPA receptor (Sommer *et al*., 1991; Burnashev *et al*., 1992b). In contrast, we observe poor editing of both GluR5 and GluR6 in cultured hippocampal neurons from E18 embryos. One possibility is that the cultured hippocampal neurons, in contrast with the cultured cerebellar granule neurons, are not in an environment that is appropriate for editing to proceed to its full extent. For instance, pyramidal neurons *in vitro* will never receive the appropriate dentate granule neuron afferences which might contribute to further differentiation and hence to higher editing of GluR5 and GluR6 KA receptors. Indeed, Mackler & Eberwine (1993) have shown that in the organized network of the hippocampal slice, the GluR5 mRNAs of individual CA1 pyramidal neurons are fully edited. In conclusion, and in agreement with the observations of Paschen *et al*. (1997), we find the extent of GluR5 and GluR6 editing *in vitro* to be particularly dependent on the developmental stage of the cells and cell dissociation, plating and culture conditions, and to differ in most cases from *in vivo* observations.

Functional implications

DRG neurons express KA receptors (Agrawal & Evans, 1986; Huettner, 1990), and it has been suggested that GluR5 is a major component of these receptors (Sommer *et al*., 1992). The subunit requirements for the expression of functional KA receptors (Herb *et al*., 1992; Sommer *et al*., 1992) and our results showing essentially unedited GluR5 in the developing DRG, suggest that in this tissue, functional KA receptors result from either unedited homomeric GluR5 subunits or the association of unedited GluR5 and KA-2 shown to be abundant in the developing DRG. In contrast, in the mature DRGs, the absence of KA-2, KA-1 (Herb *et al*., 1992) and GluR6 (present results) linked to the expression of similar amounts of edited and unedited GluR5 mRNAs (present results) suggest that functional KA-gated receptors are either homomeric non-edited or heteromeric edited and unedited GluR5 receptor channel complexes. In agreement with our results, the *I–V* relationships described by Huettner (1990) in dissociated DRG neurons resemble those of heteromeric edited and unedited GluR5 receptors. At this point, only recordings linked to single cell PCR (Lambolez *et al*., 1992) will reveal whether DRG neurons express both the unedited and edited subunits, or whether some neurons exclusively express the unedited or edited variants. Recently, this issue has been addressed in single cultured hippocampal neurons from E17–18 rat embryos, where the GluR5 and GluR6 mRNAs (but not GluR7, KA-1 or KA-2) were occasionally co-expressed, predominantly in the unedited form, although edited and unedited variants were found to coexist in the same cell (Ruano *et al*., 1995). Our results obtained with the E18 hippocampus as well as with the cultured hippocampal neurons (dissected out from 18-day-old embryos) showing low levels of GluR6 and more so of GluR5 editing are in agreement with such findings.

Conclusion

Our results suggest independent modulation of GluR5 and GluR6 Q/R editing. While in most tissues, GluR6 Q/R editing appears more efficient than GluR5, the opposite holds true in a few cases showing that Q/R editing of GluR6 is not systematically favoured as a result of a structural bias of its pre-mRNA. This suggests either that GluR5 and GluR6 are generally not coexpressed and thus are independently edited, or that distinct molecular mechanisms are involved in regulating GluR5 and GluR6 Q/R editing even when they are expressed in the same cells. The exon–intron duplex structures involved in Q/R editing of the GluR5 and GluR6 pre-mRNAs share features that are distinct from the distantly related GluR-B, and it has been proposed that GluR5 and GluR6 pre-mRNAs are natural substrates for the double-stranded RNA adenosine deaminase DRADA (Herb *et al*., 1996). However, other related enzymes have been implicated in GluR editing (RED1) and characterized in the brain (RED2) (Maas *et al*., 1996; Melcher *et al*., 1996a, 1996b; Maas *et al*., 1997), and it is becoming apparent that these enzymes have preferential targets (Herb *et al*., 1996; Maas *et al*., 1996). The study of the cellular distribution and activity of the double-stranded RNA adenosine deaminases in different cell types during ontogeny, in relation with GluR expression and editing, will be of particular interest. Another important issue will be the identification of the factors that modulate the expression and activity of the double-stranded RNA adenosine deaminases in neural cells of the developing and adult nervous system, and in neuropathologies.

Acknowledgements

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Abbreviations

CNS, central nervous system; DRG, dorsal root ganglion; KA, kainate; NR, nitrate reductase; PB, phosphate buffer; Q, glutamine residue; R, arginine residue; RT-PCR, reverse transcription-coupled polymerase chain reaction.

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