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Expression profile of AMPA receptor subunit mRNA in single adult rat brain and spinal cord neurons in situ

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Abstract

The differential expression of AMPA receptor subunit mRNA was analyzed in the adult rat brain and spinal cord using quantitative RT-PCR with laser capture microdissection. The expression of all four AMPA receptor subunits was demonstrable, with the mRNA expression level for GluR2 being the highest in all the brain areas and neuronal subsets examined. Both the absolute expression level of GluR2 mRNA and its expression relative to the other subunits were the lowest in motoneurons. The unique AMPA receptor expression profile of motoneurons may render them selectively vulnerable to AMPA receptor-mediated excitotoxicity.

Keywords: AMPA receptor; GluR2; Excitotoxicity; Quantitative RT-PCR

1. Introduction

Among the family of ionotropic glutamate receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors have attracted considerable attention because of their role not only in mediating rapid synaptic excitatory transmission in the central nervous system, but also in slow excitotoxicity (Rothstein et al., 1993; Nakamura et al., 1994; Carriedo et al., 1995; Kwak and Nakamura, 1995; Carriedo et al., 1996). There is ample evidence that AMPA receptor-mediated excitotoxicity mediates selective motoneuron loss in sporadic amyotrophic lateral sclerosis (ALS) (Ince et al., 1998; Takuma et al., 1999; Kawahara et al., 2004a). Indeed, motoneurons are reported to be particularly vulnerable to AMPA receptor agonists, both in vitro (Rothstein et al., 1993; Carriedo et al., 1996; Bar-Peled et al., 1999; Vandenberghe et al., 2000) and in vivo (Nakamura et al., 1994; Kwak and Nakamura, 1995). It has been suggested that an elevation in Ca^{2+} influx through Ca^{2+}-permeable AMPA receptors might be the mechanism by which these effects occur (Carriedo et al., 1996; Greig et al., 2000; Van Den Bosch et al., 2000).

Functional AMPA receptors are homo- or heterooligomeric assemblies that are composed of four subunits, i.e., GluR1, GluR2, GluR3 and GluR4, arranged in various combinations (Hollmann and Heinemann, 1994). The Ca^{2+} conductance of AMPA receptors differs markedly depending on whether the GluR2 subunit is a component of the receptor. AMPA receptors that contain at least one GluR2 subunit have low Ca^{2+} conductance, while those lacking a GluR2 subunit are Ca^{2+} permeable (Hollmann et al., 1991; Verdoorn et al., 1991; Burnashev et al., 1992, 1995; Swanson et al., 1997). Thus, the relative abundance of GluR2 among the AMPA receptor subunits in a particular neuron could very well determine that neuron's overall Ca^{2+} permeability and, by extension, its vulnerability to excitotoxicity.

Most studies to date that have examined the relative abundance of AMPA receptor subunits in single neurons

Abbreviations: ALS, amyotrophic lateral sclerosis; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; Cbl, cerebellum; Cx, cerebral cortex; DF, dorsal funiculus; DG, spinal dorsal gray matter; Granule, cerebellar granule cells; Motor, spinal motoneurons; Purkinje, cerebellar Purkinje cells; Sensory, spinal dorsal horn neurons in the substantia gelatinosa; SpW, spinal ventral and dorsal funiculi; VF, ventral funiculus; VG, spinal ventral gray matter; Wx, cerebral white matter

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have been carried out using cultured cells (Lambolez et al., 1996; Brorson et al., 1999; Greig et al., 2000; Vandenberghe et al., 2000; Alsbo et al., 2001; Dai et al., 2001; Tsuzuki et al., 2001), the latter instances of which utilize immature neurons. There is clear evidence that the expression of AMPA receptor subunits is developmentally regulated (Pellegrini-Giampietro et al., 1992; Jakowec et al., 1995; Pickard et al., 2000; Kumar et al., 2002), though no findings have been reported regarding their expression in single adult neurons. Therefore, it is of some urgency to examine the expression of these receptors in adult neurons since they may have a crucial role in the pathophysiology of adult human nervous system diseases such as ALS. Measuring changes in the proportion of the AMPA receptor subunits in frozen adult brain and spinal cord samples would also allow a comparison of the expression levels between different brain regions and neuronal subsets without interference from potential culture-induced changes in the AMPA receptor profile (Taubenfeld et al., 2002). Using real-time RT-PCR on laser-captured single neurons – a method that we have reported yields reproducible results when human material is used (Kawahara et al., 2003) – we analyzed the expression profile of AMPA receptor subunits in different neuronal subsets in the rat brain and spinal cord. We were specifically interested in determining whether spinal motoneurons were unique in terms of their AMPA receptor expression profile, a finding that might explain their preferential vulnerability to AMPA receptor-mediated excitotoxicity in ALS (Kawahara et al., 2003, 2004a).

2. Materials and methods

2.1. Spinal cord and brain samples

Adult male Fischer (10–12 weeks old, $n = 14$) rats and Wistar (10–14 weeks old, $n = 14$) rats for the spinal cord tissue analysis were used in this study. Animals were anesthetized deeply using diethyl ether inhalant prior to being decapitated, after which their spinal cord and brain were quickly removed and frozen in liquid nitrogen; samples were stored at −80 °C until use.

2.2. Harvesting of tissue samples

Frozen spinal cord and brain sections (100 μm) were cut with a cryostat (Model HM500 O; MICROM, Walldorf, Germany) and the resultant sections attached to glass slides. The lumbar spinal cords were dissected into ventral gray matter (VG), dorsal gray matter (DG), ventral funiculus (VF) and dorsal funiculus (DF) regions, and the cerebral samples were separated into cortical gray (Cx) and white (Wx) matter; cerebellar samples (Cbl) were also collected. All of these dissections were carried out in a freezing chamber (Model LD-550; TOMY, Tokyo, Japan) with the aid of a surgical microscope, with the tissue samples being collected into 1.5 ml test tubes containing 1 ml of TRIZOL reagent (Invitrogen Corp., Carlsbad, CA, USA). All tissue samples weighed <10 mg and were stored at −20 °C until use.

2.3. Single cell dissection

Single cell isolation was carried out using an excimer laser microdissection system (Hamamatua Photonics Ltd., Shizuoka, Japan) as previously described (Hashida et al., 2001; Kawahara et al., 2003). In brief, 20 μm thick frozen sections were attached to glass slides made of artificial quartz, fixed with 100% methanol for 60 s, and then stained with 0.1% toluidine blue. Thirty spinal motoneurons (Motor) and 60 cerebellar Purkinje cells (Purkinje) were dissected free and placed into test tubes containing 200 μl of TRIZOL reagent. The substantia gelatinosa (Sensory) of the spinal cord and the cerebellar granule cell layer (Granule) were dissected en bloc and similarly placed into tubes containing 200 μl of TRIZOL reagent. All samples were stored at −20 °C until use.

2.4. RNA extraction and reverse transcription

Total RNA was extracted from each tissue sample using the reagent TRIZOL according to the manufacturer's instructions. The single cell RNA extraction as well as reverse transcription procedures were carried out as previously described (Kawahara et al., 2003). Briefly, 200 μl of TRIZOL reagent containing the collected neurons were incubated at room temperature for 5 min; 40 μl of chloroform was then added to each tube, which were then shaken vigorously for 1 min. The tubes were centrifuged at 12,000 *g* for 30 min at 4 °C and the supernatants transferred to Phase-Lock Gel-Heavy Gel (Eppendorf AG, Hamburg, Germany), to which 120 μl of PCI (phenol/chloroform/isoamyl alcohol (25/24/1)) were added. The tubes were again centrifuged, this time at 12,000 *g* for 15 min at 4 °C, after which the supernatants were transferred to test tubes containing 10 μl of 3 M sodium acetate and 1 μl of a carrier mixture consisting of 0.9 μl of Ethachinmate (Nippon Gene Corp., Tokyo, Japan) and 0.1 μl of Pellet Paint co-precipitant NF (Novagen, Inc., Madison, WI, USA). We added 110 μl of 2-propanol to each tube, vigorously vortexed them, and then incubated them at −20 °C for ≥2 h. The tubes were spun for a third time (12,000 *g* for 30 min at 4 °C), after which the collected pellets were rinsed twice with 75% ethanol. The air-dried pellets were dissolved in 4 μl of RNase-free water and the RNA-containing pellet was treated with Amplification Grade DNaseI (Invitrogen). Reverse transcription was performed using Ready-to-go You-Prime First-Strand beads (Amersham Biosciences Corp., Piscataway, NJ, USA) and 0.5 μM oligo (dT) primer, in the case of tissue samples, or a Sensiscript RT Kit (QIAGEN GmbH, Hilden, Germany) with 0.5 μM oligo (dT) primer and 10 units of prime RNase inhibitor