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Distribution of glutamate receptor subunit GluR1 and GABA in human cerebral neocortex: a double immunolabelling and electron microscopic study

Abstract Specimens of human cerebral neocortex were obtained during neurosurgical operations and studied by immunocytochemistry and electron microscopy, using antibodies to the glutamate receptor subunit GluR1 and gamma-aminobutyric acid (GABA). Many GluR1-positive pyramidal neurons and fewer GluR1-positive non-pyramidal neurons were present in the cortex. Non-pyramidal neurons were more heavily labelled for GluR1 than pyramidal neurons. Most GABAergic neurons were labelled for GluR1. The white matter was unstained, except for occasional labelled neurons. This pattern of GluR1 immunostaining is similar to that in rat cerebral cortex, but is different from that in the hippocampus and amygdala, where large numbers of pyramidal or projection neurons, but few non-pyramidal or GABAergic neurons, were labelled for GluR1.

Key words GluR1 · GABA · Electron microscopy · Cerebral neocortex · Human

Introduction

Glutamate and aspartate are major excitatory neurotransmitters in mammalian brain, and glutamate receptors have been demonstrated throughout the central nervous system in rats and monkeys (Rogers et al. 1991; Blackstone et al. 1992; Hampson et al. 1992; Petralia and Wenthold 1992; Huntley et al. 1993; Martin et al. 1993; Vickers et al. 1993, 1995; Conti et al. 1994; Petralia et al. 1994a,b). They play crucial roles in central nervous function, including activity-dependent synaptogenesis during development, synaptic plasticity, learning and memory (Collingridge and Bliss 1987; Tocco et al. 1992), and may be important in the pathogenesis of neurological or psychiatric disorders, including epilepsy (Hosford et al. 1991), Huntington’s disease (Dure et al. 1991), schizophrenia (Harrison et al. 1991), Alzheimer’s disease (Cowburn et al. 1990), and excitotoxic neuronal cell death (Choi 1992). Glutamate receptors can be divided into G-protein coupled metabotropic receptors (Masu et al. 1991) and ionotropic receptors (Monaghan 1991). The ionotropic receptors have been classified according to their preferred agonists, as N-methyl-D-aspartate (NMDA) and non-NMDA types. On the basis of their pharmacology and physiology, non-NMDA receptors have been further classified into alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate and kainate subtypes (Sommer and Seeburg 1992).

The distribution of the inhibitory transmitter gamma-aminobutyric acid (GABA) in human cerebral cortex has been studied by immunocytochemistry (Schiffmann et al. 1988; Ong and Garey 1991b; Homung and De Tribolet 1994). The few studies of glutamate receptors in human cerebral cortex have focused on detection of receptor protein in Western blots (Blackstone et al. 1992; Breese and Leonard 1994; Breese et al. 1995) or light microscopic study of immunocytochemically labelled neurons (Huntley et al. 1994; Vickers et al. 1995). Electron microscopy is now needed to elucidate the synaptology of glutamate receptors, and the present study was therefore carried out in human cerebral neocortex, using double immunolabelling and light and electron microscopy, to investigate the relationship between GABAergic cells and one of the subunits of the AMPA glutamate receptor, GluR1 (Boulter et al. 1990; Keinanen et al. 1990; Nakanishi et al. 1990).

Materials and methods

Specimens and fixation

Eight specimens of human cerebral neocortex and subcortical white matter were used. The first (HS9402) was removed during...
surgery to excise a large falx meningioma in a 39-year-old woman, and consisted of a block of right frontal cortex (area 9 or 10 of Brodmann 1909) and subcortical white matter, near the edge of the tumour. The second (HS9403) was a specimen of right temporal pole (area 38) excised during hemispherectomy for a large right cerebral cyst in a 17-year-old girl. The third (HS9408), a block of area 8 and underlying white matter, was obtained during surgery in a 27-year-old man with supplementary motor cortex focal epilepsy. The fourth and fifth specimens (HS9410, HS9413) were blocks of left area 21, excised during temporal lobectomy for epilepsy in a 37-year-old woman and a 26-year-old man. The sixth specimen (HS9501) consisted of a block of right area 8, excised to remove a frontal cavernous angioma in a 32-year-old woman. The seventh specimen (HS9502) was from right area 45 or 46, during surgery to remove a frontal lobe tumour in a 51-year-old woman. The eighth (HS9504) consisted of a block of left inferior temporal gyrus (area 20), excised to remove a left temporal lobe glioblastoma. Specimens were fixed by immersion in a solution of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer within seconds of removal. They were then dissected into blocks of approximately 5 mm³, and fixed overnight in fresh fixative.

Immunocytochemistry

Single immunolabelling

Blocks were sectioned at 100 μm using an Oxford Vibratome. Free-floating sections were washed in five or six 1-hourly changes of phosphate-buffered saline (PBS), immersed for 20 min in a solution of 1% (w/v) non-fat dry skimmed milk in PBS (PBS-milk) to block non-specific binding of antibody, and incubated overnight in rabbit polyclonal antibodies to GluR1 (Petralia and Wenthold 1992; Chemicon, 1 μg/500 μl in PBS-milk). Sections were then washed in three changes of PBS, and incubated for 1 h at room temperature in a 1:200 dilution of biotinylated goat anti-rabbit IgG (Vector), followed by three changes of PBS to remove unreacted secondary antibody. Sections were then reacted for 1 h at room temperature with an avidin–biotinylated horseradish peroxidase complex. The reaction was visualised by treatment for 5 min in 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB) solution in TRIS buffer containing 0.05% hydrogen peroxide. The colour reaction was stopped with several washes of TRIS buffer, followed by PBS. Sections were mounted on glass slides and lightly counterstained with methyl green before coverslipping. Control sections were incubated with PBS instead of primary antibody; they showed a complete absence of immunostaining.

Double immunolabelling

Double immunolabelling was carried out using a similar protocol to that previously described (Ong et al. 1995). Briefly, free-floating sections were immunolabelled with rabbit primary antibody to GABA (Seratec, diluted 1:2000 in PBS-milk), as described for single labelled sections. On completion of the DAB reaction, sections were briefly inspected by light microscopy, and washed for a further 3 h to remove traces of unreacted antibody. They were then immunostained using anti-GluR1 primary antibodies (Petralia and Wenthold 1992; Chemicon, 1 μg/100 μl in PBS-milk). Detection was carried out using the biotinylated secondary antibody–avidin–biotinylated horseradish peroxidase method described above, except that tetramethylbenzidine (TMB) was used as the chromogen (Llewellyn-Smith et al. 1993; Ong et al. 1995). Sections were immersed for 20 min in a pre-incubation solution consisting of 10 ml of 0.1 M sodium phosphate buffer (pH 6.0), 500 μl of 1% ammoni-

Fig. 1 Light micrograph of a GluR1-immunostained section through right area 45 or 46 (HS9502). Arrows indicate densely labelled non-pyramidal neurons; arrowheads indicate moderately densely labelled pyramidal neurons. Scale bar represents 170 μm