Immunocytochemical localization of the epilepsy-related Lgi1 in rat cortical neurons

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INTRODUCTION

The leucine-rich, glioma-inactivated 1 (LGI1) gene is mainly expressed in neurons, with little or no expression detected in glial cells [1,2]. Interestingly, mutations of the LGI1 gene [1,3] have been found in patients affected by autosomal dominant lateral temporal epilepsy, an inherited epileptic syndrome characterized by auditory auras and/or other symptoms suggesting a lateral temporal origin of seizures. Despite the clinical relevance of the Lgi1 protein, information on its function(s) and/or location in neuronal cells is sparse and inconsistent. Schulte & coll. [4] provided evidence that the Lgi1 protein is associated with the presynaptic, rapidly inactivating Kv1 potassium channel suggesting its involvement in the control of the inactivation of this channel; Fukata & coll. [5] showed that Lgi1 selectively binds to a postsynaptic membrane receptor protein, thereby potentiating synaptic AMPA currents in hippocampal slices. The above hypotheses, however, are exclusively based on biochemical/biomolecular studies; therefore, precise localization of the Lgi1 protein in normal neurons and nervous tissue would help to ground sound hypotheses on its physiology. To this aim, the subcellular localization of Lgi1 was analyzed in normal rat cortex by combining biochemistry, immunohistochemistry and immunoelectron microscopy using two different anti-Lgi1 antibodies.

MATERIALS AND METHODS

Two commercially available antibodies raised against different Lgi1epitopes were used, the goat polyclonal sc-9583 antibody directed against a C-terminal peptide (Santa Cruz) and the rabbit polyclonal Ab30868 antibody (AbCam) directed against the amino acid sequence from 200 to 300. For biochemical analyses, the cortical part of two Wistar adult rat brains was grounded in liquid nitrogen and then homogenized in the presence of protease inhibitor mixture by using an Ultraturrax disperser. A post-nuclear supernatant was prepared to obtain a crude membrane fraction and a cytosolic fraction. Protein concentration was determined. Tissue fractions were separated on 10% SDS-polyacrylamide gels and then electroblotted onto nitrocellulose membrane. Destained membranes were incubated with the primary antibody: the Lgi1 immunocomplex was detected by enhanced ECL reagent (Roche), whereas 70 kDa neurofilament (mouse monoclonal antibody from Abcam) and beta III tubulin (rabbit polyclonal antibody from Abcam) were detected by BCIP/NBT solution (Sigma-Aldrich). N-deglycosylation assay was performed by using the N-glycosydaseF deglycosylation kit (Roche). For light immunohistochemistry, two Wistar rats were anaesthetised and then perfused with 4% paraformaldeyde in 0.1 M phosphate buffer, pH 7.4. After paraffin-embedding, the brains were cut into 7-µm-thick sections, which were processed with the anti-Lgi1 antibodies, revealed by biotinylated secondary antibodies (ABC

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kit, Vectastain; Vector Laboratories Inc.) For immunoelectron microscopy, two further Wistar rats were used: after perfusion, the temporal lobes were removed and either embedded in LRWhite resin or postfixed in 2.5% glutaraldehyde and 2% paraformaldehyde, then in 1% OsO4 and embedded in Epon 812. Both LRWhite- and Epon-embedded samples were then processed for immunocytochemistry using the anti-Lgi1 antibodies revealed by secondary gold-conjugated antibodies (Jackson ImmunoResearch Laboratories).

RESULTS AND CONCLUSIONS

Both anti-Lgi1 antibodies identified a single band of about 60 kDa in the whole cell homogenate, which was enriched in the microsomal fraction, whereas no bands were found in the cytosolic fraction. The incubation of the microsomal fraction with N-deglycosidase results in a shift of the Lgi1 band to an apparent molecular mass of about 55 kDa, indicating that Lgi1 is N-glycosylated in brain neurons. The antibodies against cytoskeletal components showed that the 70 kDa neurofilament was only present in the microsomal fraction, whereas beta III tubulin was found in both microsomal and cytosolic fractions. At light microscopy, the two anti-Lgi1 antibodies gave the same immunolabelling pattern in cortical neurons: the signal was specifically located in the cytoplasm, both in the soma, in dendrites, and in axons. The fine intracellular localization allowed by electron microscopy demonstrated that Lgi1 molecules occur in the rough endoplasmic reticulum (where protein synthesis occurs), in the Golgi complex (where proteins undergo glycosylation), and in the cytoplasm, where the immunolabelling mainly occurs in close proximity of neurotubules and neurofilaments, thus suggesting a possible association with cytoskeletal structures. This association is especially evident in axons, where neurotubules and neurofilaments form prominent bundles responsible for the axonal transport and shape maintainance.

Explanation of the presence of Lgi1 labelling associated with neurofilaments and neurotubules is manifold. First, Lgi1 molecules may be transported from the cell body through the axon by motor proteins. If this is the case, however, it is not clear what the final target of this anterograde transport would be, given the lack of substantial Lgi1 labelling at synapses. Second, a role for Lgi1 as a carrier in the axonal flux could be envisaged. Lgi1 structure shows regions implicated in protein-protein interactions [6], thus suggesting a function as an adaptor protein connecting motor proteins with a specific cargo consisting of soluble proteins, possibly released at or before entering the pre-synapsis. Finally, Lgi1 may be involved in the regulation of cytoskeletal organization: the axonal cytoskeleton is a dynamic structure that is constantly remodelled to ensure axon maintenance and plasticity. These dynamic changes are particularly important during development in neuronal migration and neurite outgrowth. Accordingly, LGI1 gene is intensely expressed in migratory neurons of the cortical plate at the end of the embryonic development [7]. Thus, the association of Lgi1 and axonal cytoskeletal structures may be related to the maintenance of normal structure and/or function of axons of mature neurons and, in developing neurons, with neuronal cell migration and axonal outgrowth.

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