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Original article

Developmental changes in KCNQ2 and KCNQ3 expression in human brain: Possible contribution to the age-dependent etiology of benign familial neonatal convulsions

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Abstract

Several mutations of *KCNQ2* and *KCNQ3* are considered to be associated with benign familial neonatal convulsions (BFNC). BFNC is characterized by seizures starting within several days of life and spontaneous remission within weeks to months. KCNQ channel is a heteromeric voltage-dependent potassium channel consisting of KCNQ2 and KCNQ3 subunits. To clarify the age-dependent etiology of BFNC, we examined the developmental changes in KCNQ2 and KCNQ3 expression in human hippocampus, temporal lobe, cerebellum and medulla oblongata obtained from 23 subjects who died at 22 gestation weeks to adulthood. Formalin-fixed and paraffin-embedded specimens were used for immunohistochemistry. Unique developmental changes in KCNQ2 and KCNQ3 were found in each region. A high expression of KCNQ2 was identified in the hippocampus, temporal cortex, cerebellar cortex and medulla oblongata in fetal life, but such expression decreased after birth. The expression of KCNQ3 increased in late fetal life to infancy. Simultaneous and high expressions of KCNQ2 and KCNQ3 were observed in each region from late fetal life to early infancy, coinciding with the time when BFNC occurs. Such coexpression may contribute to the pathogenesis of BFNC.

Keywords: Epilepsy; KCNQ; M-current; Benign neonatal convulsions; Neuropathology

1. Introduction

Epilepsy is a common neurological disorder afflicting 1–2% of the general population worldwide. The pathogenesis of epilepsy has a genetic component and the phenotypes often show age-dependence. Genetic abnormalities have been identified recently in age-dependent familial epilepsy syndromes [1,2]. However, the etiology of such age-dependency of epilepsy remains mostly unclear.

Benign familial neonatal convulsion (BFNC) is an age-dependent familial epilepsy syndrome characterized by clusters of generalized seizures exclusively afflicting neonates, with spontaneous remission [3,4]. Mutations in the genes encoding two subunits of KCNQ channels, *KCNQ2* and *KCNQ*3, have been identified as causes of BFNC [1,5–8]. However, the exact pathogenic mechanisms of age-dependent onset and spontaneous remission of BFNC remain to be elucidated.

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The KCNQ channel is a voltage-dependent potassium channel. Potassium current generated by KCNQ channels is called M-current. The M-current was first described in sympathetic neurons [9] and later recorded in mammalian brain neurons [10,11]. Hence, it is currently known to have a major influence on neuronal excitability governing the responsiveness of neurons to incoming inputs.

The KCNQ channel is a heteromeric tetramer consisting of KCNQ2 and KCNQ3 subunits [12]. KCNQ2 and KCNQ3 are widely expressed mainly in the hippocampus, neocortex and cerebellar cortex, presenting a specific regionalized distribution [13–16]. In these regions, KCNQ2 and KCNQ3 are co-expressed and co-assembled [15], and thus both subunits are thought to fully function when they are assembled as a hetero-tetramer, because KCNQ2/KCNQ3 heteromeric channels generate 15-fold larger current than homomeric channels [6,12–15,17–20]. The developmental pattern of KCNQ2 and KCNQ3 subunits may contribute to the age-dependent etiology of BFNC. To our knowledge, there is no information about the development of the subunits in human brain. Therefore, we investigated the developmental changes in KCNQ2 and KCNQ3 expression in the hippocampus, temporal lobe, cerebellum and medulla oblongata in human brain.

2. Materials and method

2.1. Western blotting

Specimens of normal human brain were used in Western blotting. Western blot analysis was performed as described previously [21]. Briefly, supernatants of cerebral cortex and cerebelli homogenates were collected after centrifugation, and the amounts of protein were assayed by the method of Bradford. Samples (50 lg/lane) were separated on a 7.5% SDS polyacrylamide gel and then electrophoretically transferred to a nitrocellulose membrane (HyBond-P; Amersham; NJ, USA). Blocking with 8% skimmed milk (over night at 4 °C) was followed by incubation of the membrane with an antibody against KCNQ2 (Santa Cruz Biotechnology, Santa Cruz, CA, dilution, 1:200) (overnight at 4 °C), and then with Bovine Anti-goat IgG-HRP (Santa Cruz Biotechnology, Santa Cruz, CA, dilution, 1:2000) for 1 h at room temperature. Bands were visualized using the ECL system (Amersham), and the chemiluminescence was detected with an autoradiography film (Hyperfilm-ECL; Amersham).

2.2. Human tissue specimen

Developmental immunohistochemical studies were conducted in 23 brains of subjects aged 22 gestation weeks (GW) to 75 years. Informed consent for postmortem examination was provided in writing by the patients or their family representatives in all cases. The causes of death in fetuses, infants and children were spontaneous abortion, cardiomyopathy, congenital heart disease and acute lymphocytic leukemia without central nervous system involvement, while those in adults were squamous cell carcinoma without central nervous system involvement and liver cirrhosis. Whole brains were fixed in 10% formalin for a few weeks. The specimens of the hippocampus, temporal lobe and basal ganglia were examined in the present study. All specimens were found to be histologically normal based on examination of hematoxylin and eosin stained sections.

2.3. Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were cut into 4-μm thick sections and immunohistochemically stained using streptavidin-biotin method with antibodies against KCNQ2 and KCNQ3. The sections were deparaffinized in xylene and then rehydrated in ethanol. Microwave irradiation was performed to retrieve the antigen. After cooling to room temperature, the endogenous peroxidases activity was quenched by methanol/H_2O_2. After three washes in tris-buffered saline (TBS), the sections were incubated with anti-KCNQ2 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, dilution, 1:100) (overnight at 4 °C), and anti-KCNQ3 antibody (Alomone Labs, Jerusalem, Israel, dilution, 1:100) (overnight at 4 °C). After three washes in TBS, the sections were incubated with the biotinylated second antibodies and peroxidase-conjugated streptavidin (Simplestain MAX-PO [goat and multi], Nichirei, Tokyo) for 1 h at room temperature. After three washes in TBS, the immunoproducts were visualized using diaminobenzidine (Nichirei, Tokyo). Finally, the sections were counterstained with hematoxylin.

2.4. Cell density analysis

The numbers of KCNQ2- and KCNQ3-positive cells were counted in each brain region examined. The density of positively-stained neurons was graded semi-quantitatively as negative (−: no neurons were stained), mild (±: less than 10% neurons were stained), moderate (+: less than 50% neurons were well stained), or marked (2+: more than 50% neurons were well stained). The antibody expression in the Purkinje cell layer was graded as −, negative; ±, a few fine puncta in the neuropil or on the cell surface; +, many positive puncta and intense bedded staining under low magnification on light microscopy.

3. Results

Table 1 summarizes the extent of immunoreactivities to KCNQ2 and KCNQ3 in the developing human hip-