Abstract

In the course of an experiment designed to identify novel genes, that control hematopoietic proliferation and differentiation, we found that a gene, previously thought to be brain specific, was also expressed in hematopoietic cells. It encodes the glutamate receptor 5 (GluR5), which belongs to the relatively large gene family of ionotropic glutamate receptors (iGluR).

The iGluRs are divided into three subfamilies. Based on their exogenous agonists, they are called AMPA, Kainate and NMDA receptors. GluR5 belongs to the Kainate subfamily, whose physiological function is to a large extent still unknown. Ionotropic glutamate receptors play an important role during brain development and synaptogenesis and, for that reason, may be involved in the context of some neurodegenerative diseases. Furthermore they seem to have an important pathological significance in ischaemic processes, such as strokes and heart attacks as well as in multiple sclerosis.

The ionotropic glutamate receptor is a cation channel, consisting of four or five subunits *in vivo*, and which is made permeable primarily for Ca^{2+} ions after ligand binding. The condition for the formation of a functional ion channel is the interaction of at least two different subunits from one subfamily.

The aim of the work was to show that electrical non-excitable hematopoietic cells could also express ionotropic glutamate receptors with a distinct biological function. Furthermore the investigations should clarify at the RNA level, which receptor variants participate in the formation of the receptor complex in hematopoietic cells. Two posttranscriptional modifications have been described for iGluRs: alternative RNA splicing and RNA editing.

By means of an RT-PCR based expression assay we found expression of AMPA/Kainate transcripts in human primary blood cells (CD34 positive and CD34 negative mononuclear blood cells) and in human established hematopoietic cell lines with erythroid (K-562, TF-1 and several TF-1 cell culture model systems) and myeloid (U-937, HL-60) backgrounds, respectively. Hence each cell displayed an specific AMPA/Kainate receptor expression patterns. Within the TF-1 model, it could be

shown that both cell culture conditions and cell mutations are coupled with distinct AMPA/Kainate receptor expression patterns.

An important posttranscriptional modification for the Kainate receptors GluR5 and GluR6 is the adenosine/inosine conversion by RNA editing within the transmembrane domain II. In contrast to mouse brain, this domain, which is responsible for ion permeability, was not edited in the examined human hematopoietic cells (K-562, stroma dependent TF-1 cell and TF-1 mutant 29A). The same observation could be made for the murine stroma cell line MS-5.

Almost all iGluR subunits are expressed in different splice isoforms. Two open reading frames were cloned from the growth-factor independent cell line TF-1 mutant 29A, the GluR5 variant GluR5-2b and an unpublished GluR7 variant, which was named GluR7c, in accord with other variants. Analysis of a genomic cosmid clone obtained after screening of a human chromosome 1-enriched library, showed, that the splice variant GluR7c is generated by alternative RNA splicing. Both human GluR7 variants could be cloned from the neuronal cell line NT-2. GluR7c is also expressed in the hematopoietic cell line U-937. A *Prosite* analysis of the isoforms GluR5-2b and GluR7c, expressed in TF-1 mutant 29A, predicteded protein kinase C (PKC) recognition motives in the cytosolic carboxy-terminal ends, instead of the casein kinase II (CKII) binding motif found in other known variants. Hence a heteromeric Kainate receptor, which contains the variants GluR5-2b and GluR7c, should couple signaling to the PKC pathway. To determine electrophysiological data for the new variant GluR7c, two-electrode voltage clamp method was used. Unfortunately, no reproducible ion current could be detected in frog oocytes.

These findings presented here should contribute to a better understanding of the signal transduction events in excitable and non-excitable cells and may be helpful for the development of new pharmaceutically active substances.