Abstract

Properties of glycine (GlyR) and $GABA_B$ ($GABA_BR$) receptors were studied in the adult rat medial nucleus of the trapezoid body (MNTB). MNTB belongs among brainstem auditory nuclei. Prevailing cell type in the MNTB is the principal cell (PC). Each PC receives two types of synaptic inputs. Excitatory input originating at contralateral anteroventral cochlear nucleus (AVCN), forms a giant glutamatergic nerve terminal, calyx of Held (CH). Inhibitory inputs are brought by glycinergic and GABAergic fibers of unclear origin. Synaptically released GABA and glycine modulate excitatory synaptic transmission in MNTB via receptors expressed by presynaptic and postsynaptic neurons. The goal of our work was to elucidate both the subcellular distribution and subunit composition of the receptors, thereby extend our knowledge of the function of the receptors in the MNTB.

We used immunohistochemical staining methods. The receptors were labelled by specific antibodies localized in the nervous tissue by means of fluorescence or electron microscopy.

The experimental work consisted of three phases. During the first phase we revealed that GlyRs in the MNTB form two distinct populations. Postsynaptic receptors form $\alpha 1 \beta_4$ heteromeric clusters on somatodendritic parts of PCs. These clusters colocalize with glycinergic endings thus tuned to mediate a fast postsynaptic inhibition. In contrast, GlyRs on CH are dispersedly distributed $\alpha 1$ homomeric receptors. GlyRs on soma of presynaptic neurons (globular bushy cells in AVCN) show a similar clustering and subunit composition profile as GlyRs on MNTB PCs. These results suggest that specific targeting of GlyR β-subunit produces segregation of GlyR subtypes involved in two different mechanisms of modulation of synaptic strength. Furthermore, we found that the density of postsynaptic GlyRs on soma of PC and the number of glycinergic boutons transiently decreased after the bilateral cochlear ablation. The results thus indicate a role of sensory signals in maintaining of inhibitory systems in the MNTB.

The second series of experiments was aimed to perform a quantitative analysis of presynaptic GlyR distribution on CH. We found that GlyRs locate at compartments responsible for the glutamate release. These include calyceal stalks and swellings. The density of GlyRs in swellings is higher than in stalks and the receptors preferentially occupy the side facing

away from the postsynaptic cell soma. Interestingly, the sites of the highest GlyR concentrations were found in swellings tightly juxtaposed with GABA/glycinergic nerve endings. Thus, the results indicate non/homogenous distribution of presynaptic GlyRs activated by glycine spillover from inhibitory fibers. We also suggest the existence of an activitydependent mechanism regulating the surface distribution of presynaptic GlyRs.

The third part of our experimental work enabled the first direct demonstration of GABA$_B$R in the MNTB. GABA$_B$R on CH and on glycinergic boutons were localized near presynaptic active zones and at extrasynaptic sites. Postsynaptic receptors were localized mainly at the postsynaptic density and at perisynaptic sites. Pre- and

postsynaptic GABA$_B$R included auxiliary subunit KCTD12. The results are consistent with the existence of presynaptic GABA$_B$ homo- and heteroreceptors regulating release of both excitatory and inhibitory neurotransmitters, and the postsynaptic desensitizing receptors mediating a phasic inhibition.