Abstract

GABA and glycine are major inhibitory neurotransmitters in the central nervous system. They act on ionotropic and metabotropic receptors that form oligomeric complexes in plasma membrane of neuronal cells. Pharmacological properties, subcellular distribution and function of GABA and glycine receptors depend on their subunit composition. The thesis was aimed to find subunit composition and physiological role of ionotropic glycine and metabotropic $GABA_B$ receptors in neurons of medial nucleus of trapezoid body, MNTB. The nucleus resides in the mammalian auditory brainstem and it is characterized by well defined excitatory and inhibitory inputs. Excitatory fibers form giant glutamatergic nerve terminals, calyces of Held, whereas inhibitory fibers form small GABA/glycinergic boutons. Both types of afferents innervate somatodenritic parts of MNTB principal neurons. The nucleus thus represents a suitable model for studying molecular and cellular mechanisms of interactions between excitation and inhibition.

Our experiments were performed using electrophysiology and immunohistochemistry methods. Patch clamp technique was used to record membrane currents and voltages from neurons in live MNTB slices isolated from rats or mice. Spontaneous and sound-evoked activity of murine MNTB neurons was recorded by in vivo juxtacellular recording of extracellular field potentials. Localization of receptors immunolabelled with specific antibodies was studied using confocal microscopy.

We found two functionally different glycine receptor populations in the rat MNTB. Postsynaptic receptors formed α1/β-containing clusters on somatodendritic domains of MNTB principal neurons, colocalizing with glycinergic nerve endings to mediate fast, phasic inhibitory postsynaptic currents. In contrast, presynaptic receptors on glutamatergic calyx of Held terminals were composed of dispersed, homomeric α1 receptors, triggering slow potentiation of glutamatergic transmission. These results suggest that specific targeting of glycine receptor β-subunit produces segregation of the receptor subtypes involved in two different mechanisms of modulation of synaptic strength. We have also revealed that normal cochlear activity is important for initiation of developmental changes in inhibitory transmitter subtype and expression level of pre- and postsynaptic inhibitory receptors in the rat MNTB.

The second part of the thesis was focused on mechanisms of action of pre- and postsynaptic $GABA_B$ receptors in the mouse MNTB. We have shown that $GABA_B$ expressed on calyces of Held control both spontaneous and sound-evoked discharges of MNTB neurons in adult mice. Results of these experiments have also disproved the idea of constitutive activity of presynaptic $GABA_B$ receptors causing low basal release probability at the calyx of Held synapse. Postsynaptic $GABA_B$ receptors expressed in

MNTB principal neurons were found to be regulating the activity of N- and P/Q-type voltage-gated Ca^{2+} channels. Subsequently, the postsynaptic action potential medium afterhyperpolarization phase mediated by opening of Ca^{2+}-activated SK channels was strongly reduced in the presence of baclofen, $GABA_B$ receptor agonist. When postsynaptic spikes were repeatedly evoked at high frequencies, such $GABA_B$ activity actually induced higher postsynaptic firing rates of MNTB neurons. In this way, postsynaptic $GABA_B$ receptors, generally considered as important regulators of basal neuronal excitability, could surprisingly increase the reliability of excitatory synaptic transmission in the mammalian MNTB.