S 13-4

FUNCTION OF GLYCINERGIC CENTRIFUGAL FEEDBACK IN DISTAL RETINAL SIGNALING

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Glycine is one of the major inhibitory neurotransmitters opening Cl^- permeable receptors. The glycinergic synapse is critical for modulation of glutamate signals in retinas. The role of glycine in the inner retina is known for inhibiting glutamate release and shaping light responses in ganglion cells. However the function of glycine in the outer retina is largely unknown. Glycinergic synapse in the outer retina is believed to be a network input via a group of centrifugal neurons, glycinergic interplexiform cells. These neurons are excited by glutamate in the inner plexiform layer (IPL), and feed back glycine to the outer plexiform layer (OPL) reversing to the glutamate pathway in retina. We investigated the role of glycine centrifugal feedback. Tiger salamander retinas were used as a model system for conducting electrophysiological and immunocytochemical studies. The effects of glycine were investigated on dark membrane potentials and light responses in photoreceptors, horizontal cells and bipolar cells in retinal slices by whole-cell gramicidin perforated recordings. We find that glycine directly excites horizontal cells and the dendrites of On-bipolar cell caused by Cl^- efflux-induced depolarization. Glycine also enhances glutamate release from photoreceptors by a combined mechanism of Cl^- efflux and internal Ca^{2+} increase. The net effect of glycine would enhance the outer retinal signaling, contrasting to the effect of glycine in the inner retina. Our immunolabeling results demonstrate that Na-K-2Cl^- cotransporters, Cl^- uptake transporter, are present in photoreceptor terminals, horizontal cells and bipolar cell dendrites, suggesting that Cl^- equilibrium potentials might be high in these neurons. Activation glycine receptors would cause an excitatory Cl^- response in the neurons. The glycinergic input in the OPL was localized by antibody labeling for glycine transporters in the axon terminals of glycinergic interplexiform cells. A strong labeling pattern of glycine transporters in the OPL corresponds to the electrophysiological results that glycinergic synapse is abundant and predominant in modulation of the outer retinal signals. Furthermore, both whole-cell recording and antibody labeling results indicate that glycine receptors and glycine feedback in the outer retina can be up-regulated by repetitive light stimulation. This study explores an excitatory action of glycine feedback in the retinal network.

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SPHINGOSYLPHOSPHORYLCHOLINE INDUCES DIFFERENTIATION OF MESENCHYMAL STEM CELLS TO SMOOTH MUSCLE CELLS VIA ACTIVATION OF G PROTEIN-COUPLED RECEPTORS

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Mesenchymal stem cells (MSCs) can differentiate into diverse cell types including adipogenic, osteogenic, chondrogenic, and myogenic lineages. In the present study, we demonstrated for the first time that sphingosylphosphorylcholine (SPC) induces differentiation of human adipose tissue-derived mesenchymal stem cells (hATSCs) to smooth muscle-like cell types. SPC increased the expression levels of several smooth muscle-specific genes, such as α-smooth muscle actin (alpha-SMA), h_1-calponin, and SM22alpha, as potent as TGF-beta1 and -beta3. SPC elicited delayed phosphorylation of Smad2 after 24 h exposure, in contrast to rapid phosphorylation of Smad2 induced by TGF-beta treatment for 10 min. Pretreatment of the cells with pertussis toxin or U0126, an MEK inhibitor, markedly attenuated the SPC-induced expression of alpha-SMA and delayed phosphorylation of Smad2, suggesting that the $G_{i/o}$-ERK pathway is involved in the increased expression of alpha-SMA through induction of delayed Smad2 activation. In addition, SPC increased secretion of TGF-beta1 through ERK-dependent pathway, and the SPC-induced expression of alpha-SMA and delayed phosphorylation of Smad2 were blocked by SB-431542, a TGF-β type I receptor kinase inhibitor, or anti-TGF-beta1 neutralizing antibody. Silencing of Smad2 expression with short interfering RNA (siRNA) abrogated the SPC-induced expression of alpha-SMA. These results suggest that SPC-stimulated secretion of TGF-beta1 plays a crucial role in SPC-induced SMC differentiation through Smad2-dependent pathway. Both SPC and TGF-beta increased the expression levels of serum response factor (SRF) and myocardin, transcription factors involved in smooth muscle differentiation. siRNA-mediated depletion of SRF or myocardin abolished the alpha-SMA expression induced by SPC or TGF-beta. These results suggest that SPC induces differentiation of hATSCs to smooth muscle-like cell types through $G_{i/o}$-ERK-dependent autocrine secretion of TGF-beta, which activates Smad2-SRF/myocardin-dependent pathway.