Abstract

Molecules called synapse organizers are involved in the formation of synapses. Among them, clustering of neurexin-1β (b) by interacting with neuroligin-1 is known to cause synaptogenesis. The ability of Neurexin-1β to induce synaptogenesis in synapse organizers has been studied from various viewpoints, and although the elucidation of this molecular function has been widely attempted, the use of this function as a molecular machine has not been quite common. In this study, I developed an engineered synapse organizer based on Neurexin-1β and established a method to fabricate a glass microelectrode in which the main body was insulated by glass and only the tip was metal (Gold). By combining these, I attempted to induce selective synaptogenesis in the glass microelectrode. The engineered synapse organizer we developed formed synapse-like structures on the surfaces of inorganic microbeads and glass microelectrodes by artificial binding, not intrinsic binding. The localization of synapsin, which acts as a synaptic marker molecule, and Rab3 in the introduced marker was consistent. The engineered synapse organizer we developed can be said to be capable of inducing synaptogenesis in the glass microelectrode. When the tips of microbeads and glass microelectrodes bearing Rab3 antibodies were contacted with neurons expressing Rab3, I confirmed the accumulation of Rab3 despite the binding by antibodies. The method for

fabricating the glass microelectrode was developed by preferentially selecting a tool that is universally used for the patch clamp method, which may be a compatible tool for fields that employ such electrophysiological measurement methods. I employed a focused ion beam (FIB) machine for the fabrication of the tip of the microelectrode, and I was able to perform highly reproducible fabrication. This allowed us to efficiently fabricate an electrode with an arbitrary tip shape using the FIB, if an electrode with gold conducting could be fabricated by a micropipette puller. Finally, in a proof-of-principle experiment using chick forebrain neurons, selective synaptogenesis was induced in the glass microelectrode in a non-CO2-dependent culture medium. Chick neurons are simple to culture, and unlike rats and other mammals, it is known that removing the forebrain neuron from an egg embryo is unlikely to cause biological problems. Experiments showed that synaptogenesis by the engineered synapse organizer took at least 4 hours, but chick forebrain neurons transfected at room temperature and open air survived for at least 8 hours and formed synapses with antibody-modified microbeads and glass microelectrodes.

Keywords: Electrophysiology, Neuron, Synapse Organizer, Glass Microelectrode, Protein Adsorption