Reply to the Comment of D. J. Woodbury

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In the previous paper (1), we reported an anion channel (94 pS) and three types of cation channels (Type 1 (250 pS), Type 2 (248 pS), and Type 3 (213 pS)) from synaptic vesicles from rat brains incorporated into planar lipid bilayers. As commented by Woodbury (2), however, we did not eliminate contamination by synaptic membrane or other nonsynaptic membrane fragments, although we checked the homogeneity of the preparation by electron microscopic observation and found more than 80% content of synaptic vesicles (Sato, M., M. Kasai, and H. Ishikawa, unpublished observation). From the experiment using lipid bilayer incorporated synaptic membranes, similar ion channels to those observed in our paper (1) have been reported by other researchers, especially the anion channel and two types of cation channels such as Types 2 and 3. It is probable that these channels may have originated from contamination by synaptic membranes rather than synaptic vesicles. However, as far as the Type 1 cation channel is concerned, there are no such reports. In the experiment where we used the synaptic vesicle fraction, we observed the Type 1 cation channel frequently, therefore it is probable that this channel originated from synaptic vesicles.

As pointed out by Woodbury (2), since the fusion of vesicles with the planar bilayer is prerequisite for these experiments, minor components of ion channels would dominantly be observed if their fusion probabilities are overwhelmingly high. We are interested in fusion activity of synaptic vesicles and studied fusion processes of synaptic vesicles and synaptic membranes with artificial liposomes. Both membrane fractions did not fuse in the presence of a few millimolar of Ca^{2+}, but both membrane fractions began to fuse to liposomes under certain conditions, for example, lowering pH (3). Fusion of synaptic vesicles was especially outstanding. This result may partly be attributable to the smaller size of synaptic vesicles. From such observations we thought that there is not a large difference between fusion activities of synaptic membrane and synaptic vesicles to the lipid bilayer, although the two systems are different from each other. It is probable that these ion channels originated from synaptic vesicles.

Woodbury and Miller (4) proposed a nystatin method to increase the fusion probability. This method is excellent because all vesicles became equally fusogenic and because the fusion frequency could be measured. They stated that the nystatin conductance disappeared after the vesicles fused with a sterol-free bilayer, and that this treatment did not affect ion channels existing in vesicles. However, there remains some anxiety about the study of new types of ion channels, since we have no idea about the effect of nystatin or sterol on such ion channels. We are studying a different method to make fusion probability equal: solubilization-reconstitution of channel proteins into uniform liposomes followed by fusion to lipid bilayers. This method was successfully applied to ion channels in fractionated sarcoplasmic reticulum membrane vesicles from rabbit skeletal muscle (5, 6). Although we applied this method to synaptic vesicles, the fusion probability was not high enough to evaluate the results at present.

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