Letters to the Editor

Making Synaptic Vesicles Fuse with Lipid Bilayers

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Dear Sir,

In a recent paper, Sato, Inoue, and Kasai (1992) reported on ion channels in synaptic vesicle membranes studied by planar lipid bilayers. Although their article clearly demonstrates the initial characterization of several ion channels, there is no evidence that the observed channels actually come from synaptic vesicles. The alternative explanation is that these channels come from contaminating membrane fragments. Unfortunately, this latter explanation is more likely because of two simplifications (flaws) in the experimental design used by Sato et al. The synaptic vesicle preparation was not pure and the vesicles were not treated to make them uniformly fusigenic.

Although it is unrealistic to expect a homogeneous vesicle preparation, the issue of purity still deserves careful evaluation. Sato et al. do acknowledge in their discussion that "the possibility of contamination of a small amount of presynaptic plasma membranes in the fraction (of) vesicles cannot be ruled out," however, they do not indicate the magnitude of this possible contamination problem. In reality, the synaptic vesicle preparation used by Sato et al. is not highly pure. They state they followed the purification "methods of Huttner et al. (1983), except that the gel filtration step was omitted." This is unfortunate because it is the gel filtration step that makes the preparation of Huttner et al. something that could be called highly pure. Without gel filtration, at least 50% of the protein in the preparation is due to contamination by large nonsynaptic vesicles and soluble proteins. This estimate is based on the data in Fig. 4 of Huttner et al., but the estimate is uncertain since protein was measured by absorbance at 280 nm and both lipids and ATP interfere at this wavelength. In my experience with synaptic vesicle purification (using a sensitive protein assay on synaptic vesicles isolated from pure cholinergic neurons), gel filtration removes over 75% of the original protein by excluding large contaminating vesicles. Thus, it seems probable that *at least* 50% of the protein in the synaptic vesicle preparation of Sato et al. is due to contaminating membrane fragments.

Even without major contamination of the synaptic vesicle preparation as presented above, all the channels reported by Sato et al. could still be due to trace contaminants. This is because there is an inherent *variation* in the ability of different vesicles to fuse with lipid bilayers. Consider, for example, a synaptic vesicle preparation that contains 99.9% synaptic vesicles that are not fusigenic with bilayers and 0.1% contaminating vesicles that are fusigenic. Following addition of 10^8 of these vesicles only some of the 10^5 contaminating vesicles fuse with the bilayer and hence only their channels are observed.

Is it reasonable to expect a difference in fusion rates for different vesicles? The answer from the literature is clearly yes. In 1988 this journal published my results on the spontaneous fusion of vesicles that were made with and without the ion channel, porin (Woodbury and Hall, 1988a). Although the majority of the porin-containing vesicles fused over the time course of the experiment, few, if any, of the porin-free vesicles fused. Similar results were also reported by Perin and MacDonald (1989). Separately, Cohen, Niles, and Akabas showed that under identical fusion conditions (600 mM formamide gradient across the bilayer) vesicles that contained nystatin channels readily fuse, whereas vesicles containing porin channels "did not result in any significant fusion" (Cohen, Niles, and Akabas, 1989). The reason for the difference in the rate or extent of fusion is clear from follow-up papers out of the same two laboratories (Woodbury and Hall, 1988b; Niles, Cohen, and Finkelstein, 1989); namely, that fusion is induced by vesicle swelling and the extent of swelling depends on the permeability properties of the channel. Thus, the presence (or absence) of channels in the vesicle membrane and the permeability properties of these channels, dramatically change the rate of fusion for each vesicle.

The only solution I know of, to avoid this dilemma of variation in vesicle fusion rate, is to treat all vesicles in the preparation in such a way as to make them equally fusigenic. Although in reality it is not possible to reach this ideal, it is now possible to come close. By treating the vesicle preparation with nystatin and ergosterol all vesicles can be made reasonably fusigenic. The original method (Woodbury and Miller, 1990) has been successfully used in other systems to greatly increase the probability of fusing in vesicles that otherwise are less fusigenic (Bear et al., 1992). My own experiments with cholinergic synaptic vesicles indicate that the vesicle fusion rate increases dramatically when the nystatin/ergosterol method is used. Hence, ion channels that Sato et al. and I (unpublished observations) observe in the bilayer due to spontaneous fusion events (non nystatin/ergosterol fusion) must come mostly from a small subpopulation of vesicles.

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In fact, without either uniformly fusigenic vesicles or 100% purified protein, all ion channels identified only by fusion of vesicles into planar membranes cannot be assigned any cellular location.

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