Calcium channel and membrane fusion activity of synexin and other members of the Annexin gene family

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Membrane fusion is a poorly understood but fundamental biological process which supports such disparate events as organelle biogenesis, cell division, and exocytotic secretion of enzymes, hormones, and neurotransmitters. In the case of exocytosis, the fusing membranes are those of secretory granules and plasma membranes; and recent biochemical, biophysical, and molecular studies have implicated the calcium binding protein synexin (Annexin VII) in the process (1). In the absence of calcium, synexin is soluble in the cytosol. However, when intracellular calcium concentration rises, synexin is recruited into the membrane. The mechanism by which calcium-activated synexin causes fusion to occur seems to involve coincident penetration of both fusing membranes by a hydrophobic synexin polymer. The lipids then cross the "hydrophobic bridge" and fusion ensues in a manner directed by the protein (1–3).

The most compelling evidence for such an intimate relationship between synexin and the membrane interior has been the observation that synexin can both sustain gating currents in membranes and bilayers (4), and exhibit voltage-dependent calcium channel activity in target membranes (5, 6). An example of such ion channel activity is shown in Fig. 1 for recombinant human synexin. Calcium ions flow through open synexin channels, but neither barium ions nor magnesium ions are conducted. This exquisite selectivity for calcium by the channel is also paralleled by the cation selectivity which synexin exhibits for both the chromaffin granule aggregation and PS liposome aggregation as well as fusion processes.

We have also recently reported that the ion channel property of synexin is shared by other members of the annexin gene family. For example, endonexin II (Annexin V) forms channels in PS bilayers formed at the tip of a patch pipet (7). Calcium is necessary, as in the case of synexin, to drive the endonexin molecule into the bilayer. However, once inserted endonexin II will transport other cations, such as lithium and cesium. Examples are shown for these ions in Fig. 2.

We have now found that yet another member of this gene family, lipocortin I (Annexin I) also forms an ion channel. As is the case for the other annexins, the lipocortin I channel conducts calcium. Furthermore, both barium and potassium can also be conducted through the lipocortin I channel. Fig. 3 shows the conduction of potassium ions through open bovine [des 1–12] lipocortin-I channels. In this particular case, two lipocortin channels are incorporated, and the relationship between the magnitude of the unitary currents and the bilayer potential reflects a linear conductance of ~50 pS for either of the channels. Furthermore, the expanded records in Fig. 3 *B* illustrate the frequent appearance of other brief conductance levels, which is a common feature of channel activity expressed by all three of the annexin channels now reported. Studies in our laboratory with full length human lipocortin I in a patch pipet arrangement give identical results (Rojas, E., H. B. Pollard, and H. Haigler, manuscript in preparation). We have also noted calcium channel activity in purified calelectrin 67 K (Annexin VI, generously supplied by Dr. Thomas Sudhof).

In contrast to the common ion channel properties of the four annexins studied to date, studies in our laboratory on fusion potency of these different annexins reveal that only synexin and full length lipocortin will direct true fusion of PS liposomes. This conclusion is based on the ANTS/DPX volume mixing assay, which depends on collisional quenching by molecules, originally in isolated vesicles. By contrast, neither endonexin II, nor calelectrin 67 K, nor calpactin II (Annexin II) support true fusion. In fact, endonexin II only "binds" to the liposome substrates, whereas calelectrin 67 K and calpactin II only support liposome "aggregation." Indeed, a form of lipocortin-I, truncated by the first 12 amino acids ([des 1–12]), loses the capacity to drive fusion, and only supports the liposome aggregation process. [des 1–12] lipocortin I also supports lipid mixing, as indicated by the octadecyl-rhodamine assay.

Because the ion channel function seems to be common to at least four members of the annexin gene family, we have further considered the possibility that the locus of the channel may be the COOH-terminal tetrad repeat common to family members (7). To this end we have recently taken advantage of the newly reported crystal structure of endonexin II in its water-soluble form (8) in order to model the structure of the tetrad repeat when in the membrane (3, 9). This model is based on the fact

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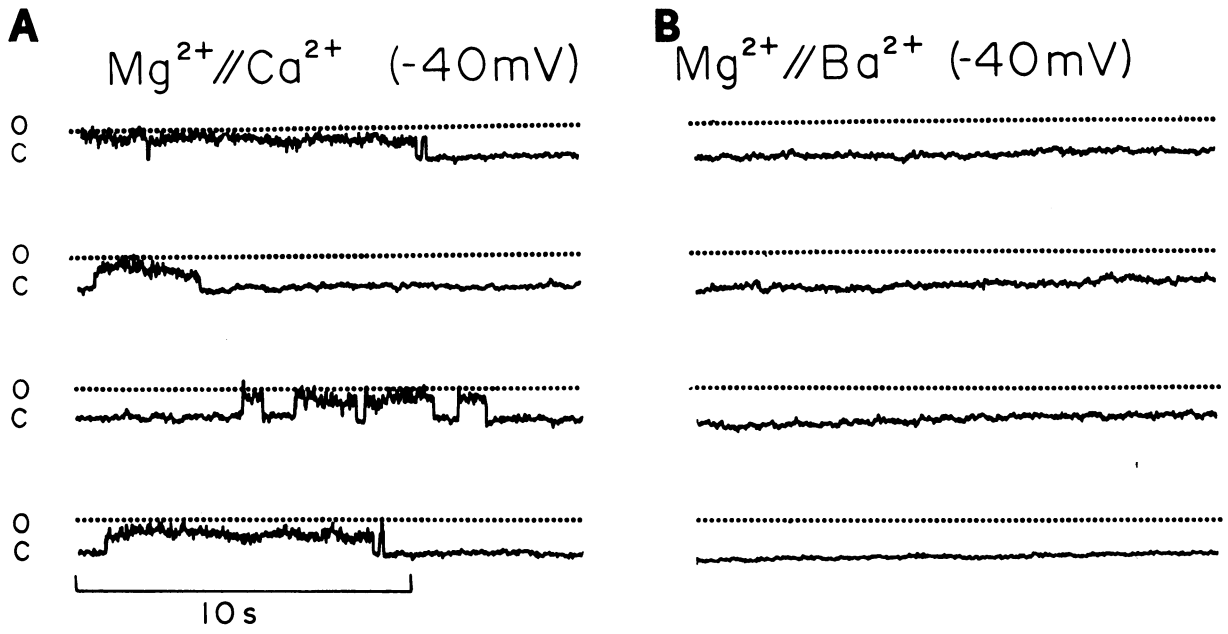

FIGURE 1 Channel activity of recombinant human synexin. Channel forming activity was incorporated into a PS bilayer formed at the tip of a patch pipet by an increase in ambient calcium concentration. (*A*) Bi-cationic system is 40 mM $CaCl_2$ in the bath (*cis*) and 40 mM $MgCl_2$ in the pipet (*trans*). The pipet voltage is −40 mV, and calcium ions flow through the open synexin channel. (*B*) Bi-cationic system now contains 40 mM $BaCl_2$ in the bath (*cis*) and 40 mM $MgCl_2$ in the pipet (*trans*). No barium current flows under conditions where the channel was quite active. See references 5 and 6 for methodological details.

that regions between the A and B helices, and D and E helices can form elements of an eight strand beta-barrel motif now thought to be common to a number of membrane resident sodium, potassium, and calcium channels (10). An image down the mouth of the channel is shown in Fig. 4, in which the different helices and beta structures are delineated by an alpha carbon backbone representation.

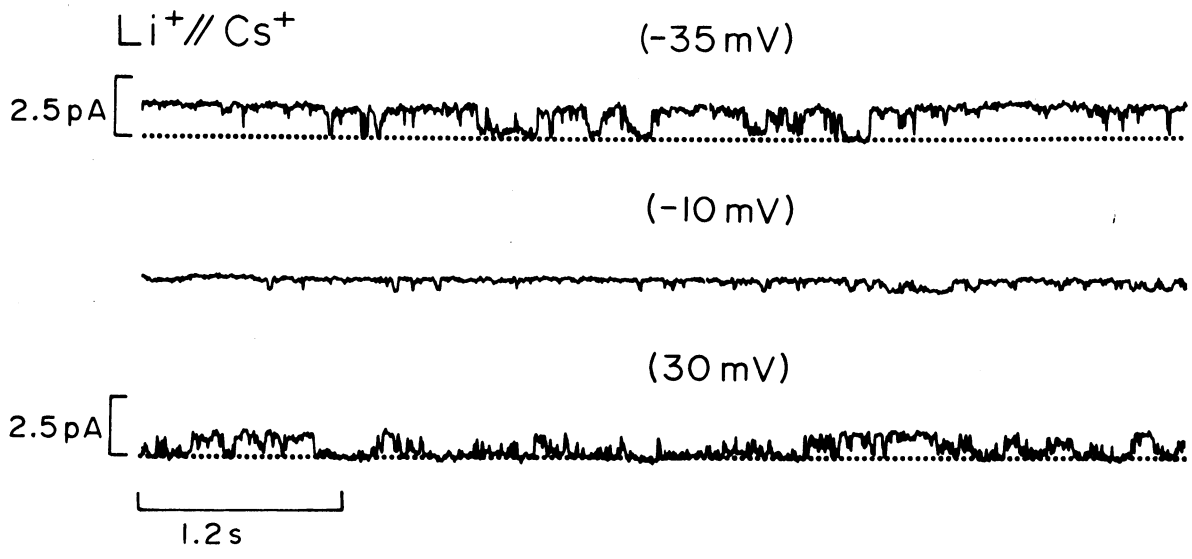

FIGURE 2 Channel activity of human endonexin II. The system is again the patch pipet with PS bilayer at the tip, and the different compartments include 200 mM LiCl in the bath (*cis*) and 100 mM CsCl in the pipet (*trans*). Dotted line in the upper record is the closed state, whereas it is the open state in the lower record. With the pipet voltage set at −35 mV (*upper trace*), Li^+ flows into the patch pipet. With a pipet voltage set at 35 mV (*lower trace*), Cs^+ flows from the patch pipet into the bath. Virtually no current flows at 0 mV. The data are from reference 7.

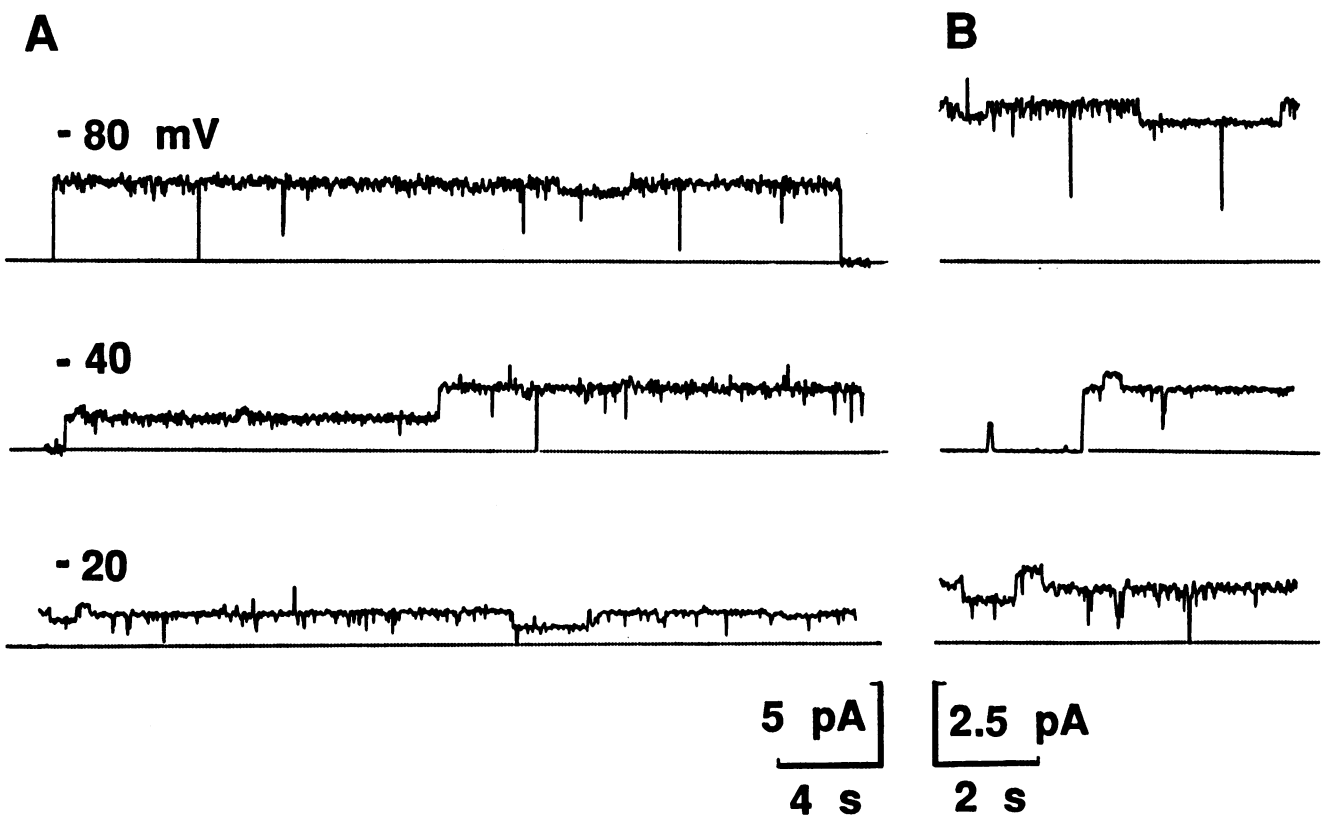

FIGURE 3 Channel activity of bovine [des 1–12] lipocortin I. The system is a planar lipid bilayer with a membrane of POPE/PS,1:1, and bovine [des 1–12] lipocortin I is incorporated after elevation of the ambient calcium concentration. The *cis* compartment contains 70 mM $BaCl_2$, whereas the *trans* compartment contains 140 mM K aspartate. (*A*) Current records obtained at different transbilayer potentials. Two lipocortin-I channels with the same conductance have been incorporated into the bilayer. The segment of record at −80 mV shows the current corresponding to the opening of an individual channel. The prevalent unitary currents at different bilayer potentials indicate a linear conductance of 50 pS. (*B*) Details of the current traces in *A*. The scales for time and current amplitude have been expanded as shown in the lower right part of the figure. Note the frequent appearance of very brief sub-conductance levels, which are a characteristic feature of the lipocortin I system. Equivalent observations have been made in a patch pipet system with human full length lipocortin I.

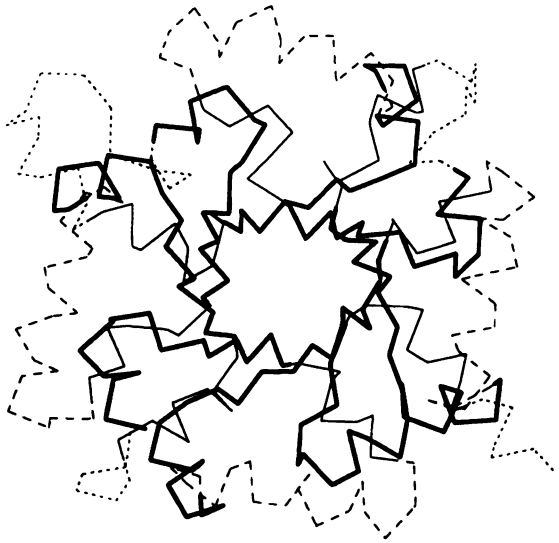

FIGURE 4 Molecular model of the annexin channel. The view is down the mouth of the channel, perpendicular to the plane of the membrane. The alpha carbons of the COOH-terminal tetrad repeat are connected to form the structure shown. The central cavity delineated in heavy lines is the beta barrel TIM structure made of the S1 and S2 segments between the A and B helices, and the B and E helices, respectively, of each of the four repeats. The radially oriented helices in heavy lines are the E and B helices. Other helices are C (*dashed lines*); and A and D (*thin lines*). The dotted lines represent connecting links between the E helix of one repeat and the A helix of the next repeat.

We conclude that the property of ion channel function is widespread in the annexin gene family, and is most likely based on the conserved COOH-terminal domain. By contrast, the capacity to drive membrane fusion is limited to synexin and lipocortin I, and is therefore most likely controlled, as least in part, by sequences associated with the respective unique NH_2-terminal domains. The physiologic importance of the ion channel function in any annexin remains unknown, and the relationship of channel function to the mechanism of membrane fusion presently rests on the structural information explicit in the facts of channel existence. These facts indicate that annexin molecules are clearly able to traverse the membrane, and we presently favor the hypothesis that synexin and lipocortin I may take advantage of this fact to drive membrane fusion. We also appreciate that interactions between membranes occur in a continuum, ranging from simple adherence to complete fusion, and that different members of the annexin gene family may prove to be differentially important in promoting these and related processes.

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