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Inositol 1,4,5-Trisphosphate-gated Channels in Cerebellum: Presence of Multiple Conductance States

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The mechanism by which inositol 1,4,5-triphosphate ($InsP_3$) induces calcium (Ca) release from the reticulum of canine cerebellum was examined. Reticular membrane vesicles used in these experiments accumulated Ca in the presence of ATP and then released ~30% of the accumulated Ca upon addition of micromolar concentrations of $InsP_3$. When these membrane vesicles were incorporated into planar lipid bilayers, $InsP_3$-gated Ca channels were observed. Up to four current amplitudes were observed at a given voltage, yielding conductances of 20, 40, 60, and 80 pS with 50 mM Ca as the current carrier. Thus, the cerebellar $InsP_3$-gated Ca channel exhibits four conductance levels that are multiples of a unit conductance step. Moreover, examination of the single-channel records showed both openings directly to each of the current levels and rapid transitions between current levels. These four conductance steps may reflect the interaction among the four $InsP_3$ receptors thought to comprise the $InsP_3$-gated Ca channel in these tissues. Examination of the $InsP_3$ dependence of channel openings and Ca release from vesicles, however, yielded Hill coefficients of 1–1.3. Thus, we hypothesize that it takes only one molecule of $InsP_3$ to open the channel. The observation that the conductance of the $InsP_3$-gated Ca channel assumes four levels that are multiples of a unit conductance suggests that the number of interacting $InsP_3$ receptors in one complex can vary from one to four and supports the hypothesis that the channel is a tetramer.

The phosphatidylinositol cascade plays an important role in the mechanism by which several hormones and neurotransmitters increase intracellular calcium (Ca) concentration (Streb et al., 1983; Abdel-Latif, 1986; Berridge and Irvine, 1989). The basic mechanism involves receptor activation of phospholipase C, with the subsequent hydrolysis of plasmalemmal phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol. $InsP_3$ is then thought to open a Ca channel in the endoplasmic reticulum, resulting in Ca release from this internal Ca store, and hence an increase in intracellular Ca concentration.

Purkinje cells of mammalian cerebellum contain a high density of $InsP_3$ receptors (Mignery et al., 1989; Ross et al., 1989), much higher than that measured in any other cell type to date (Worley et al., 1987; Marks et al., 1990; Mourey et al., 1990). Using specific antibodies to the $InsP_3$ receptor, intracellular membranes in all regions of the Purkinje cell were shown to contain the receptor (Mignery et al., 1989; Ross et al., 1989).

Recently, an $InsP_3$ receptor from cerebellum was purified (Supattapone et al., 1988; Maeda et al., 1990) and cloned (Furuichi et al., 1989; Mignery et al., 1989). The reconstituted receptor was shown to support $InsP_3$-induced Ca fluxes from vesicles (Ferris et al., 1989, 1990) and to form Ca-permeable channels in planar lipid bilayers (Hingorani et al., 1990; Maeda et al., 1991). These biochemical and molecular studies add support to the hypothesis that the receptor is an ion channel. The $InsP_3$ receptor from aortic smooth muscle has also been purified (Chadwick et al., 1990). In both aortic and cerebellar preparations, the purified receptor was shown to be a tetramer (Supattapone et al., 1988; Chadwick et al., 1990; Maeda et al., 1990, 1991; Mignery and Sudhof, 1990). Recently, the primary structure and biochemical properties of the $InsP_3$ receptors from brain and smooth muscle have been reported to be very similar (Marks et al., 1990). It has been suggested that (at least for rat basophilic leukemia cells) $InsP_3$-induced Ca release is highly cooperative [Hill coefficient greater than 3 (Meyer et al., 1988, 1989)], suggesting interaction between the $InsP_3$ receptors within the complex. This type of interaction would impose stringent requirements for inducing Ca release from intracellular stores and may be important for regulation of synaptic release from cerebellar neurons.

The present study was undertaken to determine the properties of the $InsP_3$-gated channel from cerebellum and to test if interaction among $InsP_3$ receptors is evident at the single-channel level. Specifically, reticular membrane vesicles from cerebellum were studied using a Ca release assay and planar lipid bilayer techniques for an analysis of the function of $InsP_3$-gated Ca channels.

Materials and Methods

Membrane vesicles were isolated from canine cerebellum using a differential centrifugation procedure (Watras and Benevolensky, 1987). Briefly, cerebellum (excised from anesthetized dogs) was minced, and then homogenized with a Brinkman Polytron in 4 vol of buffer A (5 mM NaN_3/100 μM EGTA/20 mM HEPES, pH 7.4). Another 4 vol of buffer A were added to the homogenate, and the suspension was centrifuged for 20 min at 4000 × g_{max} (Beckman 35 Ti rotor). The supernatant fluid was filtered through two layers of cheesecloth, and the filtrate was centrifuged for 30 min at 90,000 × g_{max} (Beckman 60 Ti rotor). The pellet from the latter spin was resuspended in buffer B (0.6 M KCl/5 mM NaN_3/20 mM $Na_4P_2O_5$/10 mM HEPES, pH 7.2) to a final concentration of 4 ml/gm cerebellum and centrifuged for 20 min at 4000 ×

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g_{max} (Beckman 60 Ti rotor). The resulting supernatant fluid was centrifuged for 30 min at 63,000 × g_{max} (Beckman 60 Ti rotor). The pellet from this last spin was resuspended in buffer C [10% sucrose, 10 mM 3-[N-Morpholino]propanesulfonic acid (MOPS), pH 7.0] and either frozen in liquid nitrogen (and stored at −80°C) or used immediately for reconstitution experiments.

Ca uptake and release by the membrane vesicle preparations were monitored by dual-wavelength spectrophotometry, using the Ca indicator antipyrylazo III (Watras and Benevolensky, 1987). Briefly, calcium uptake was initiated by addition of membrane vesicles (20 μl) to a cuvette containing reaction media [final volume, 450 μl; final concentrations, 0.4 mg/ml membrane vesicles, 100 mM KCl, 1.5 mM Na_2ATP, 0.5 mM $MgCl_2$, 10 mM creatine phosphate, 8 U/ml creatine phosphokinase, 5 mM NaN_3, 400 μM antipyrylazo III, 20 mM HEPES (pH 6.8, 25°C)]. The calcium uptake was monitored using a Johnson Research Foundation dual-wavelength spectrophotometer (720 nm, 790 nm), and then at the time indicated, Ca release was initiated by addition of 1–5 μl $InsP_3$ (final concentration as specified in figure captions). Total Ca content of the vesicles was determined by addition of 1 μl 1 mM Ca ionophore A23187. Each experiment was calibrated by three serial additions of 2 μl of 0.5 mM $CaCl_2$. Total Ca concentrations in the reaction media and in the membrane preparations were measured by atomic absorption spectroscopy and were shown to be 4.5 μM Ca in reaction media and 25 nmol Ca/mg in reticular membrane vesicles.

Channel incorporation into planar lipid bilayers was accomplished as outlined previously (Smith et al., 1986; Ehrlich and Watras, 1988), except that a KCl gradient was used for vesicle fusion. Final solutions used for channel analysis were as described previously [*cis* solution: 250 mM HEPES–Tris, 1 mM EGTA, 0.5 mM $CaCl_2$ (0.1 μM free Ca), pH 7.3; *trans* solution: 250 mM HEPES, 53 mM $Ca(OH)_2$, pH 7.3]. In these experiments, *cis* refers to the cytoplasmic side of the channel and *trans* refers to the lumenal side of the channel. All compounds (e.g., $InsP_3$) were added to the *cis* chamber.

Channel insertion and subsequent experiments were monitored under voltage-clamp conditions with a pair of Ag/AgCl electrodes contacting the solutions via CsCl junctions. The channel currents were amplified using a patch-clamp amplifier (Yale model MK-5, Warner Instruments, Hamden, CT) and recorded on chart (General Scanning, Watertown, MA) and tape recorders (Dagan Corp., Minneapolis, MN). Data were filtered with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) to 2000 Hz, transferred to a personal computer (digitized at 5 kHz), and analyzed using pCLAMP version 5.5 (Axon Instruments, Foster City, CA).

All reagents were of analytical grade, and deionized water was glass distilled prior to use. The $InsP_3$ was purchased from Calbiochem. Lipids were purchased from Avanti Polar Lipids, Birmingham, AL.

Results

$InsP_3$-induced calcium release from vesicles

Before incorporating cerebellar reticular membrane vesicles into planar lipid bilayers for analysis of $InsP_3$-gated Ca channels, the vesicles were assayed for $InsP_3$-induced Ca release using the Ca indicator antipyrylazo III. As shown in Figure 1, cerebellar membrane vesicles accumulate Ca in the presence of ATP and then rapidly release Ca upon addition of 7 μM $InsP_3$. The Ca ionophore A23187 was then added to release the remaining intravesicular Ca. The extent of $InsP_3$-induced Ca release amounted to 30% of the accumulated Ca.

$InsP_3$-gated calcium channels

Consistent with the observation that $InsP_3$ induces Ca release from cerebellar membrane vesicles, this membrane preparation was shown to contain $InsP_3$-gated Ca channels after the vesicles were incorporated into planar lipid bilayers. In the absence of $InsP_3$, current steps were rare or nonexistent in bilayers containing cerebellar membranes (Fig. 2, top three traces). Addition of 2 μM $InsP_3$ to the *cis* chamber, however, resulted in transient increases in membrane current (Fig. 2, bottom three traces) corresponding to the openings of $InsP_3$-gated Ca-permeable channels. The maximum open probability observed was <15%

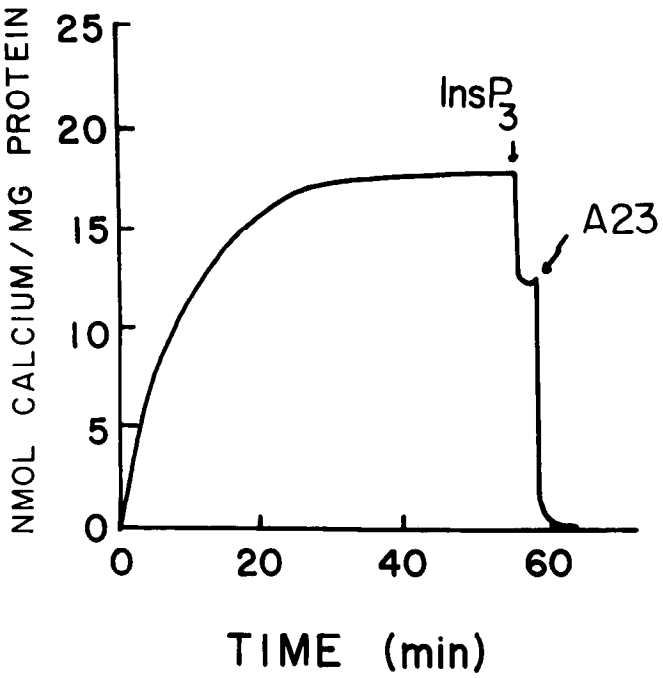

Figure 1. Time course of Ca uptake and $InsP_3$-induced Ca release from cerebellar reticular membrane vesicles. Conditions were as described in Materials and Methods. *$InsP_3$* (6 μM) was added as indicated. *A23* represents the addition of the Ca ionophore A23187 (2 μM).

despite using optimal concentrations of $InsP_3$ (see below), adenine nucleotides (Ehrlich and Watras, 1988; Ferris et al., 1990), and Ca (Bezprozvanny et al., 1991). In addition, transitions between the open and closed state of the channel were rapid, with a mean open time less than 10 msec.

Even with the compressed time scale used in Figure 2, it appears that when the channel was open the current amplitudes were not uniform as expected for single-channel events. Further examination of the $InsP_3$-activated currents using an expanded time scale gave the same result. After addition of $InsP_3$, up to four current levels were observed at every potential tested. The channel openings at a holding potential of −50 mV are shown in Figure 3 because at this holding potential, the larger driving force for calcium makes it easier to distinguish all four current levels.

Transitions between current steps also occur, with current increasing, for example, from level two to level three, followed by an abrupt return to zero current (bottom trace of Fig. 3). The presence of concerted openings to each of the four current levels, coupled with the transitions between these current amplitude levels, suggests that the $InsP_3$-gated Ca channel has four conductance levels.

To show that the observed current levels were multiples of a unit step, we determined the current–voltage relationship of the cerebellar $InsP_3$-gated Ca channel (Fig. 4*A*). The open triangles represent channel openings to the level with the longest duration that allows the most reliable estimate of the current amplitude at all voltages. The slope conductance was calculated to be 60 pS. Assuming that this represented the third of four conductance steps, broken lines corresponding to conductances of 20, 40, and 80 pS were drawn (Fig. 4*A*). These predicted conductances are a reasonable fit to the measured currents (Fig. 4*A*). The deviation from the predicted line for level four may be due to the small number of events observed at this level.

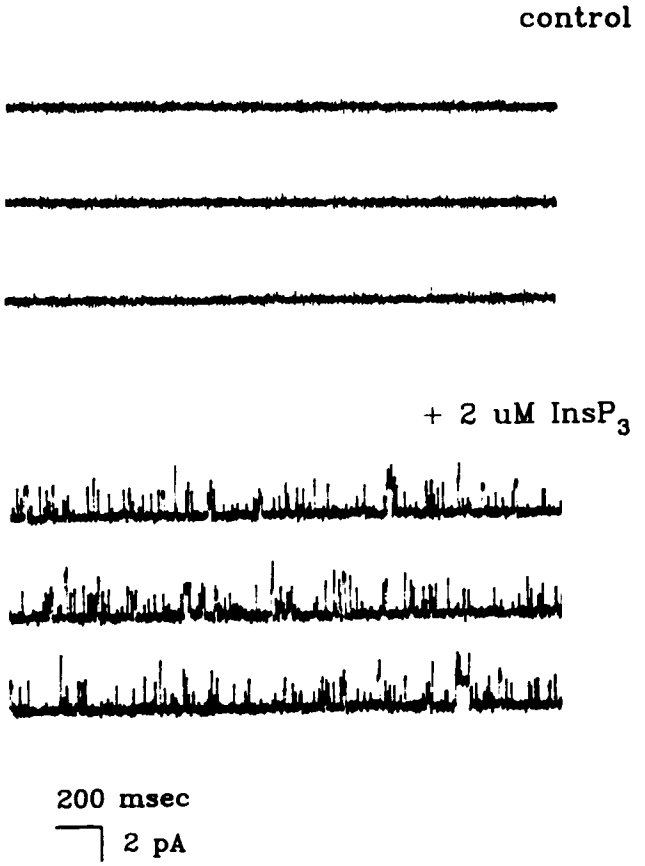

Figure 2. $InsP_3$ activation of Ca-permeable channels from cerebellar reticular membranes incorporated into planar lipid bilayers at a compressed time scale. In the absence of $InsP_3$, current jumps were not detected (*top three traces*). Channel openings were observed as upward deflections from the baseline after addition of 2 μM $InsP_3$ to the cytoplasmic, or *cis*, side of the membrane (*bottom three traces*). ATP (0.5 mM) was present on the cytoplasmic side of the membrane in this experiment to increase open probability of the $InsP_3$-gated channels (Ehrlich and Watras, 1988; Ferris et al., 1990). The holding potential was 0 mV.

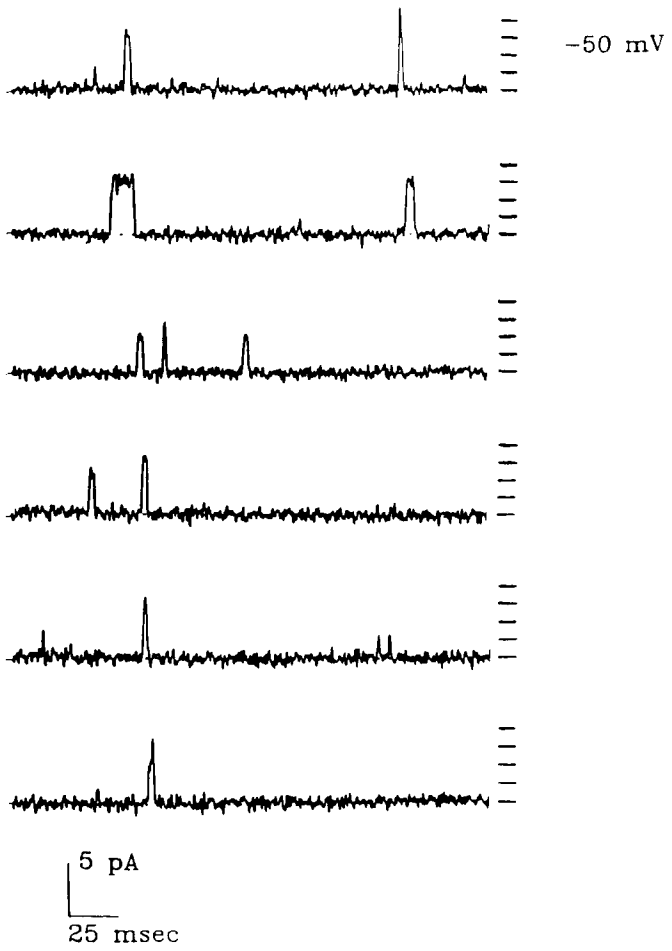

Figure 3. $InsP_3$-gated channels from cerebellum incorporated in planar bilayer are shown at expanded time scale: channel openings at a holding potential of −50 mV after addition of 2 μM $InsP_3$. Channel openings are shown as *upward deflections* from zero current. *Short lines in right margin* of records indicate unit steps in current.

The current steps from aortic $InsP_3$-gated channels reported earlier (Ehrlich and Watras, 1988) also could be fit by the conductances generated from the cerebellar data. A comparison of the current–voltage relationships of the $InsP_3$-gated channels from the cerebellar and aortic preparations is shown in Figure 4*B* for the lowest current step. This 20 pS conductance step in the cerebellar preparation was indistinguishable from that in the aortic preparation. The conductance of this channel was originally assigned the value of 10 pS (which represents the slope conductance from −20 to 0 mV; Ehrlich and Watras, 1988). Over a broader range of voltages (−50 to 0 mV), however, the data yield a conductance of 20 pS.

An amplitude histogram of the channel currents (measured at −50 mV) is shown in Figure 5. The mean current amplitudes were 1.7, 3.5, 4.9, and 6.6 pA, representing 29%, 35%, 35%, and 1% of the channel events, respectively. Thus, the current amplitudes are multiples of ~1.6 pA (at −50 mV), with openings to the first three current levels more prevalent than openings to the fourth level. The mean open time to level three (2.7 msec) is longer than the mean open time for levels one and two (1.0 and 1.6 msec, respectively), suggesting that the channel is more stable at level three.

One explanation for the appearance of four conductance states in bilayer experiments is the incorporation of four independent channels. Assuming n independent channels opening randomly, and using a binomial distribution, we expect that the probability of openings to level k (denoted P_k) would be

$$P_k = c_n^k p^k (1 - p)^{n-k}, \quad (1)$$

where C_n^k is the binomial coefficient. Given a maximum open probability (P) of the channels of 10% and $n = 4$, we expect the probability of openings to the fourth, third, and second levels would be in the ratio of 3.4×10^{-4}, 1.2×10^{-2}, and 0.16 if normalized to the probability of openings to the first level. The distribution obtained in our experiments (see above) is very different from that predicted assuming four independent channels, suggesting that the four receptors interact within the tetrameric channel complex.

The $InsP_3$-gated channels from cerebellum were only weakly voltage dependent over the voltage range tested (0 to −50 mV). A 43 mV change was needed to obtain an e-fold change in the open probability, suggesting that 0.6 effective charges must cross the electric field to open the channel. By comparison, the voltage-dependent sodium channel requires a change of 4 mV to obtain an e-fold change in the open probability (i.e., six charges

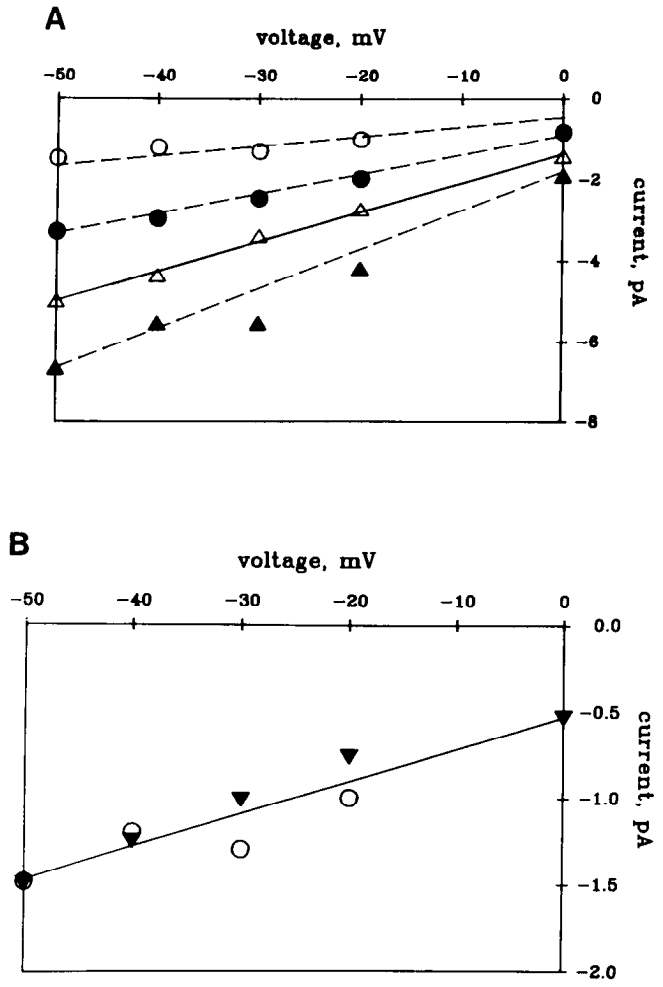

Figure 4. Current–voltage relationship of the $InsP_3$-gated channel. *A*, Channels from cerebellum exhibited four current amplitudes at each voltage. *Points* represent the measured currents (mean of current amplitude). The *solid triangles* represent means of only 3–10 openings to the fourth level at each voltage. The *solid line* was calculated by linear regression on the points represented as *open triangles*, and the *broken lines* are calculated assuming that the conductance calculated from the *open triangles* is the third level. *B*, Comparison of the lowest current steps from aorta (▼; taken from Ehrlich and Watras, 1988) and cerebellum (○). The *solid line* was calculated by linear regression of the average of all values at each voltage. Conductance is 20 pS.

must move to open the channel; Hille, 1984). The value of 0.6 for the $InsP_3$-gated channel of cerebellum is similar to the weak voltage dependence of the $InsP_3$-gated channel from beet vacuole (Alexandre et al., 1990) and aortic smooth muscle (Ehrlich and Watras, 1988) but differs from the highly voltage-dependent ($InsP_3$-*in*dependent) calcium channels in pancreatic endoplasmic reticulum (Schmid, 1990).

$InsP_3$ dependence of calcium release and channel opening

The observation of multiple conductance steps suggests an interaction among the four $InsP_3$ receptors that comprise the channel complex. We speculated that this interaction would also be reflected in the $InsP_3$ dependence of Ca release or Ca channel opening. Specifically, the $InsP_3$ dependence of $InsP_3$-induced Ca release was expected to exhibit positive cooperativity, with a Hill coefficient of 3–4, as demonstrated in permeabilized rat basophilic leukemia cells (Meyer et al., 1988, 1989).

Figure 6*A* shows the $InsP_3$ dependence of Ca release from

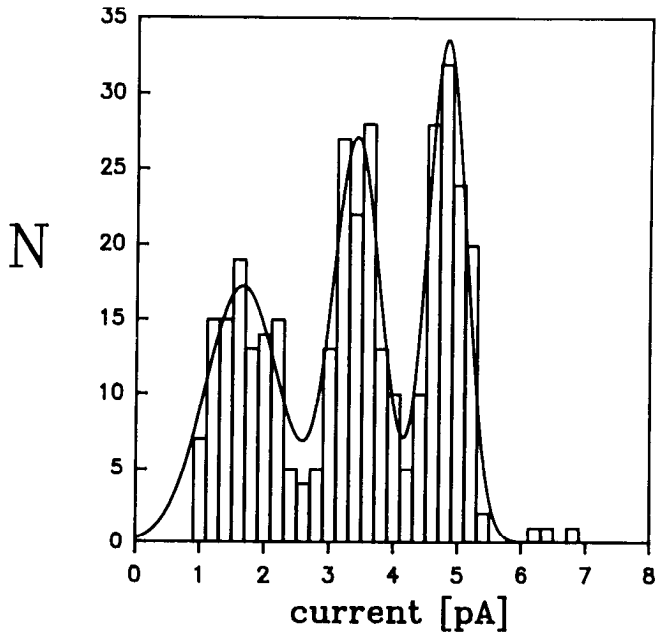

Figure 5. Amplitude histogram of the $InsP_3$-gated channel. The amplitudes of all current jumps of duration longer than 1 msec were accumulated over a 55 sec recording at the holding potential of −50 mV (350 events). The first three peaks were fit by Gaussian curves and yielded peaks at 1.7, 3.5, and 4.9 pA. In this time period, there were only three openings to the fourth level, with a mean amplitude of 6.6 pA.

cerebellar membrane vesicles. Half-maximal $InsP_3$-induced Ca release was observed at 0.15 ± 0.01 μM $InsP_3$ with Hill coefficient of 1.3. As an estimate of the rate of $InsP_3$-induced calcium release, the percentage of calcium released 3 sec after addition of $InsP_3$ was also assessed. The later data yielded an EC_{50} value of 0.16 μM $InsP_3$ with Hill coefficient of 1.2 (data not shown).

The $InsP_3$ dependence of channel opening was also measured for channels from cerebellar membranes incorporated in bilayers (Fig. 6*B*). In this analysis, a threshhold of 1 pA (at 0 mV transmembrane potential) was used to calculate the open probability of the channel at each $InsP_3$ concentration. This threshhold includes all channel openings to levels two, three, and four. At a holding potential of 0 mV, it is difficult to distinguish openings to level one from the baseline current noise. It was not possible, however, to determine the $InsP_3$ dependence at transmembrane voltages high enough to distinguish level one from the noise, because we could not maintain membranes for long periods of time at these voltages. Half-maximal probability of channel openings was observed at 0.2 μM $InsP_3$. Similar to the Ca release data, the open probability data yielded a Hill coefficient of 1.0. These data suggest that binding of only one molecule of $InsP_3$ is necessary to open the $InsP_3$-gated Ca channel.

Discussion

In this article, we described some functional properties of $InsP_3$-gated channels from cerebellum. We have shown that $InsP_3$ induces Ca release from cerebellar reticular membrane vesicles and $InsP_3$ opens channels that have been incorporated into planar lipid bilayers. More interestingly, we have shown that the channel has four conductance levels that are multiples of a unit step of 20 pS. Because we could not fit the data assuming four independent channels, we concluded that the channel openings

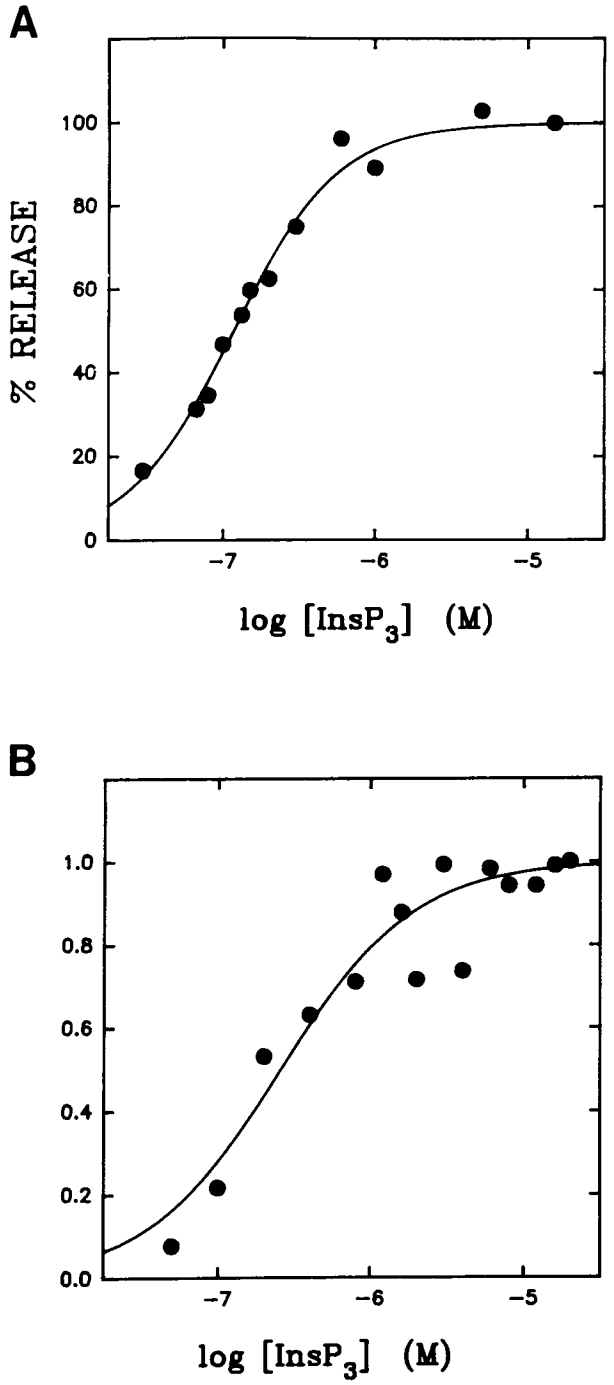

Figure 6. $InsP_3$ dependence of Ca release and channel opening. *A*, $InsP_3$ dependence of Ca release from cerebellar reticular membrane vesicles. $InsP_3$-induced Ca release was initiated 7 min after initiation of Ca uptake (as described in Materials and Methods). Data have been normalized to Ca release at 15 μM $InsP_3$; *100* corresponds to the release of 23 ± 1% of the accumulated Ca. *B*, $InsP_3$ dependence of cerebellum Ca channel activity in the bilayer. Data were normalized; *1.0* corresponds to an open probability of 14%, the maximum open probability observed in this experiment.

to the different levels represent the interactions of subunits in the tetrameric channel complex. Nonetheless, the Hill coefficients for both Ca release and for channel openings are ~1.0, suggesting that a single molecule of $InsP_3$ is necessary to activate the channel complex. We present below a model that may account for the apparent discrepancy between the findings that the single-channel currents are not independent and that only a single molecule of $InsP_3$ is needed to open the channel.

Two groups have shown that $InsP_3$ activated a high-conductance calcium channel in rat brain membranes (Vassilev et al., 1987; Ashley, 1989). This channel appears to be similar to the ryanodine-sensitive Ca release channel in skeletal muscle sarcoplasmic reticulum described by Smith et al. (1986). Opening of the ryanodine-sensitive Ca release channel by $InsP_3$, however, is controversial [compare results of Ehrlich and Watras (1988) and Suarez-Isla et al. (1988)]. Several groups have not seen $InsP_3$-dependent activation of the ryanodine-sensitive calcium channel in either cardiac (Rousseau et al., 1986) or skeletal muscle (Penner et al., 1989; Ehrlich and Watras, 1988; Vilveen and Coronado, 1988; Palade et al., 1989; Valdivia et al., 1990) preparations. There was no evidence in the present study of $InsP_3$ activation of the ryanodine-sensitive calcium channel in cerebellar membrane vesicles. This is not to say that the cerebellar preparation was devoid of ryanodine receptor; recent reports demonstrated the presence of ryanodine receptors in brain (McPherson and Campbell, 1990) and cerebellum (Ellisman et al., 1990). Moreover, specific ryanodine binding was detected in the cerebellar membranes used in the present study (B_{max} = 0.13 pmol/mg, K_D = 3 nM; R. Klonoski and J. Watras, unpublished observations), and high-conductance channels were observed infrequently in the bilayer (Bezprozvanny et al., 1991). This high-conductance channel appears to be in low density in aortic and cerebellar membranes, especially when compared to the density of the $InsP_3$ receptor, and is distinct (both in terms of pharmacology and electrophysiology) from the $InsP_3$-gated calcium channel (Bezprozvanny et al., 1991).

In a previous report, we indicated that $InsP_3$ activated a low-conductance Ca channel in sarcoplasmic reticulum isolated from aortic smooth muscle (Ehrlich and Watras, 1988). The single-channel records from the aortic membrane vesicle preparation in the previous report showed transitions between two or three current levels (Ehrlich and Watras, 1988), which we interpreted at the time as being due to the presence of two or three independent $InsP_3$-gated Ca channels in the planar lipid bilayer. Based on our current results and our initial observations using purified receptor (Hingorani et al., 1990), the different channel amplitudes in those recordings may instead represent interaction among $InsP_3$ receptors comprising the channel complex. Indeed, multiple conductance levels were first identified using the purified receptor (Hingorani et al., 1990). Examination of the single-channel recordings in the present study clearly shows defined channel openings to each of the four current levels and also shows transitions between current levels (Figs. 2, 3). Such concerted openings and closings, coupled with the observed transitions between current levels, strongly suggest the presence of a single channel with multiple conductance steps. More quantitative evidence that the channel openings are not independent is obtained from the frequency distribution of the current amplitudes (Fig. 5). If there were four channels opening independently, there would be a large number of openings to the unit level and decreasing numbers of channel openings to the second, third, and fourth levels assuming open probabilities of about 10% as seen in our experiments. In addition, openings to subsequent levels would predominantly occur sequentially, in steps. The representative current recordings and the amplitude histogram show that channel openings to the first three levels are equally common and that the openings occur in a concerted manner, rather than in sequential steps.

Recently, the $InsP_3$ dependence of Ca release from rat basophilic leukemia cells was shown to be highly cooperative, exhibiting a Hill coefficient greater than 3 (Meyer et al., 1988, 1989). The authors speculated that binding of $InsP_3$ to three or four receptors comprising the tetrameric complex is necessary to open the Ca channel. This, however, does not appear to be the situation in cerebellar membrane vesicles. In cerebellar membrane vesicles, the $InsP_3$ dependence of Ca release showed Hill coefficients of 1.3–1.5 (present results; Volpe et al., 1990). Similarly, the $InsP_3$ dependence of channel opening yielded a Hill coefficient of 1.0, as did the $InsP_3$ dependence of $InsP_3$ binding (Worley et al., 1987). Thus, we hypothesize that binding of only one $InsP_3$ molecule is necessary to open the Ca channel.

The reason for the difference in Hill coefficients between the present study and that described for rat basophilic leukemia cells is not clear. The discrepancy may reflect tissue differences, but other explanations exist. For example, the majority of the measured cooperativity occurs when Ca release is $<10\%$ of the maximum (Meyer et al., 1989). If it is assumed that low levels of Ca release represent a low open probability of the channel, we would need to measure the open probability of the channel at $<10\%$ of the maximum. Because the maximum open probability is $<15\%$, it would be very difficult for us to quantitate channel properties reliably at these low levels of activity. Another explanation presented by Iino (1990) suggests that the cooperativity seen in rat basophilic leukemia cells is attributable to changes in free-Ca concentration. The latter conclusion is based on the observation that increases in free-Ca concentration up to 300 nM can markedly increase the extent of $InsP_3$-induced calcium release from permeabilized smooth muscle cells (Iino, 1990). Thus, if the Ca concentration is not maintained constant, Ca released from the endoplasmic reticulum at a given $InsP_3$ concentration may exert positive feedback on the $InsP_3$-gated Ca channel, resulting in even further/faster Ca release. This complicates analysis of the $InsP_3$ dependence of Ca release and raises the possibility that the cooperativity of $InsP_3$-induced Ca release reported previously (Meyer et al., 1989) may be due in part to the Ca dependence of $InsP_3$-induced Ca release, especially because in the experiments with the rat basophilic leukemia cells, the free Ca concentration did not appear to be controlled with Ca chelators. Recently, we have examined the effects of Ca on the cerebellar $InsP_3$-gated Ca channel, and consistent with the results of Iino (1990) and Finch et al. (1991), we observed a marked Ca-dependent activation of the $InsP_3$-gated Ca channel over the Ca concentration range 0.05–0.3 μM (Bezprozvanny et al., 1991). Similar measurements are necessary to determine if the $InsP_3$-gated Ca channel in rat basophilic leukemia cells responds like the cerebellar $InsP_3$-gated Ca channel.

The model that we propose to explain the data relies on the observations that the purified $InsP_3$ receptor from both cerebellum and aortic smooth muscle exists as a tetramer (Supattapone et al., 1988; Chadwick et al., 1990; Maeda et al., 1990, 1991). This tetramer has a pinwheel appearance, with the four $InsP_3$ receptors radiating from a central point (Chadwick et al., 1990). We hypothesize that the conductance of a single $InsP_3$ receptor is 20 pS, though interactions with an adjacent $InsP_3$ receptor will increase the overall conductance by 20 pS steps (yielding four conductance levels in the tetramer: 20, 40, 60 and 80 pS). According to this model, four identical receptors are arranged in a ring such that activation of one receptor promotes the activation of a neighboring $InsP_3$ receptor. One molecule of $InsP_3$ can easily open one, two, or three conducting pores, with some favoring activation of three pores simultaneously. Activation of the fourth channel may require binding of a second molecule of $InsP_3$. This model is consistent with biochemical and structural findings.

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