# Isoform-Specific Function of Single Inositol 1,4,5-Trisphosphate Receptor Channels

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ABSTRACT The inositol 1,4,5-trisphosphate receptor ($InsP_3R$) family of $Ca^{2+}$ release channels is central to intracellular $Ca^{2+}$ signaling in mammalian cells. The $InsP_3R$ channels release $Ca^{2+}$ from intracellular compartments to generate localized $Ca^{2+}$ transients that govern a myriad of cellular signaling phenomena (Berridge, 1993. *Nature.* 361:315–325; Joseph, 1996. *Cell Signal.* 8:1–7; Kume et al., 1997. *Science.* 278:1940–1943; Berridge, 1997. *Nature.* 368:759–760). Most cells express multiple $InsP_3R$ isoforms, but only the function of the single type 1 $InsP_3R$ channel is known. Here the single-channel function of single type 2 $InsP_3R$ channel is defined for the first time. The type 2 $InsP_3R$ forms channels with permeation properties similar to that of the type 1 receptor. The $InsP_3$ regulation and $Ca^{2+}$ regulation of type 1 and type 2 $InsP_3R$ channels are strikingly different. Both $InsP_3$ and $Ca^{2+}$ are more effective at activating single type 2 $InsP_3R$, indicating that single type 2 channels mobilize substantially more $Ca^{2+}$ than single type 1 channels in cells. Furthermore, high cytoplasmic $Ca^{2+}$ concentrations inactivate type 1, but not type 2, $InsP_3R$ channels. This indicates that type 2 $InsP_3R$ channel is different from the type 1 channel in that its activity will not be inherently self-limiting, because $Ca^{2+}$ passing through an active type 2 channel cannot feed back and turn the channel off. Thus the $InsP_3R$ identity will help define the spatial and temporal nature of local $Ca^{2+}$ signaling events and may contribute to the segregation of parallel $InsP_3$ signaling cascades in mammalian cells.

## INTRODUCTION

The $InsP_3$ receptor gene family encodes three homologous $InsP_3$-binding proteins with three recognized domains (i.e., $InsP_3$ binding, regulatory/coupling, and channel; Mignery and Südhof, 1990, 1993; Südhof et al., 1991; Blondel et al., 1993). The ligand-binding and channel domains are highly conserved. The least conserved domain is the regulatory/coupling domain, which contains several potential regulatory sites (including a $Ca^{2+}$-binding region; Mignery et al., 1992; Sienaert et al., 1996). The regulatory/coupling domain also physically links the $InsP_3$-binding and channel domains. The relatively high heterogeneity of the regulatory domain suggests that interactions between the three domains may be isoform specific. This implies that the function of the homotetrameric $InsP_3R$ channels may be heterogeneous. Because heterotetrameric $InsP_3R$ channels may also exist (Monkawa et al., 1996; Joseph et al., 1996), it is even more likely that $InsP_3R$ channels may indeed be functionally heterogeneous.

To test this possibility, the function of the different $InsP_3R$ channel isoforms must be defined. A great deal is already known about type 1 $InsP_3R$ single-channel function (reviewed in Bezprozvanny and Ehrlich, 1995). However, very little is known about the single-channel properties of the other two $InsP_3R$ channel isoforms. One obstacle has been the difficulty in defining a reliable way to isolate homogeneous receptor populations. Recently, our laboratory established that a essentially homogeneous population of type 2 $InsP_3R$ channels could be isolated from ventricular cardiac myocytes (Perez et al., 1997). This provided the means to perform the first head-to-head functional evaluation of two different $InsP_3R$ channel isoforms.

## MATERIALS AND METHODS

### Materials

Inositol 1,4,5-trisphosphate was purchased from LC Laboratories (Woburn, MA). Heparin was purchased from Fluka Chemical Corp. (Ronkankoma, NY). 3-[(3-Cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) was from Boerhinger Mannheim Biochemicals (Indianapolis, IN). L-α-Phosphatidylcholine, L-α-phosphatidylethanolamine, and L-α-phosphatidylserine were obtained from Avanti Polar Lipids (Pelham, AL).

### Membrane preparation, sucrose gradient sedimentation, and reconstitution

Microsomal membranes from bovine cerebellum and acutely isolated ferret ventricular cardiac myocytes were prepared as described previously (Mignery et al., 1990; Perez et al., 1997). Microsomes were solubilized in 1% CHAPS and fractionated on 5–20% sucrose gradients. Gradient fractions containing the highest levels of receptor protein were identified by Western blotting and then reconstituted into proteoliposomes as described previously (Mignery et al., 1992; Perez et al., 1997).

### Planar lipid/protein bilayer formation

Planar lipid bilayers were formed across a 220-μm-diameter aperture in the wall of a Delrin partition as described (Perez et al., 1997). Lipid bilayer-forming solution contained a 7:3 mixture of phosphatidylethanolamine and phosphatidylcholine dissolved in decane (50 mg/ml). Proteoliposomes were added to the solution on one side of the bilayer (defined as *cis*). The other side was defined as *trans* (virtual ground). Standard solutions (unless otherwise specified) contained 220 mM $CsCH_3SO_3$ *cis* (20 mM *trans*), 20

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mM HEPES (pH 7.4), and 1 mM EGTA ($[Ca^{2+}]_{FREE}$ = 250 nM). The $[Ca^{2+}]_{FREE}$ was verified by using a $Ca^{+2}$ electrode. A custom current/voltage conversion amplifier was used to optimize single-channel recording. Acquisition software (pClamp; Axon Instruments, Foster City, CA), an IBM-compatible 486 computer, and a 12-bit A/D-D/A converter (Axon Instruments) were used. Single-channel data were digitized at 5–10 kHz and filtered at 1 kHz. Channel sidedness was determined by $InsP_3$ sensitivity. The orientation of the channels studied was such that the $InsP_3$-sensitive side (i.e., cytoplasmic side) was in the *cis* compartment.

## RESULTS

Type 1 $InsP_3R$ receptor was isolated from bovine cerebellum. The type 2 $InsP_3R$ was obtained from isolated ventricular cardiac myocytes. Microsomes enriched in either type 1 or type 2 $InsP_3Rs$ were CHAPS solubilized and then fractionated on 5–20% linear sucrose gradients. Tetrameric $InsP_3R$-containing fractions were identified by Western blotting and reconstituted into phosphatidylcholine:phosphatidylserine (3:1) proteoliposomes (Perez et al., 1997). Immunoblotting revealed that each proteoliposome population contained essentially one type of $InsP_3R$ (data not shown). These proteoliposomes were then incorporated into planar lipid bilayers.

Incorporation of proteoliposomes into bilayers revealed $InsP_3$-sensitive, heparin-blocked $Ca^{2+}$ channels (Fig. 1 *A*). These channels were insensitive to ryanodine. The $InsP_3$ and heparin acted only from one side of the channel (presumably the cytoplasmic side). Both type 1 and type 2 channels were characterized by frequent fast opening events, with few opening events lasting longer than a few milliseconds. The unitary $Ca^{2+}$ (70 pS) and $Cs^+$ (280 pS) conductances of the type 1 and type 2 channels were very similar (Fig. 1, *B* and *C*). This suggests that different types of $InsP_3R$ channels have similar permeation properties.

It is well established that cytoplasmic $InsP_3$ and $Ca^{2+}$ regulate single type 1 $InsP_3R$ channels (Bezprozvanny et al., 1991; Watras et al., 1991). The type 1 channel is activated by micromolar $InsP_3$ only over a narrow range of cytoplasmic $Ca^{2+}$ concentrations. In this study the $InsP_3$ and $Ca^{2+}$ sensitivities of the type 2 $InsP_3R$ channel were defined for the first time. A monovalent cation ($Cs^+$) was used as a

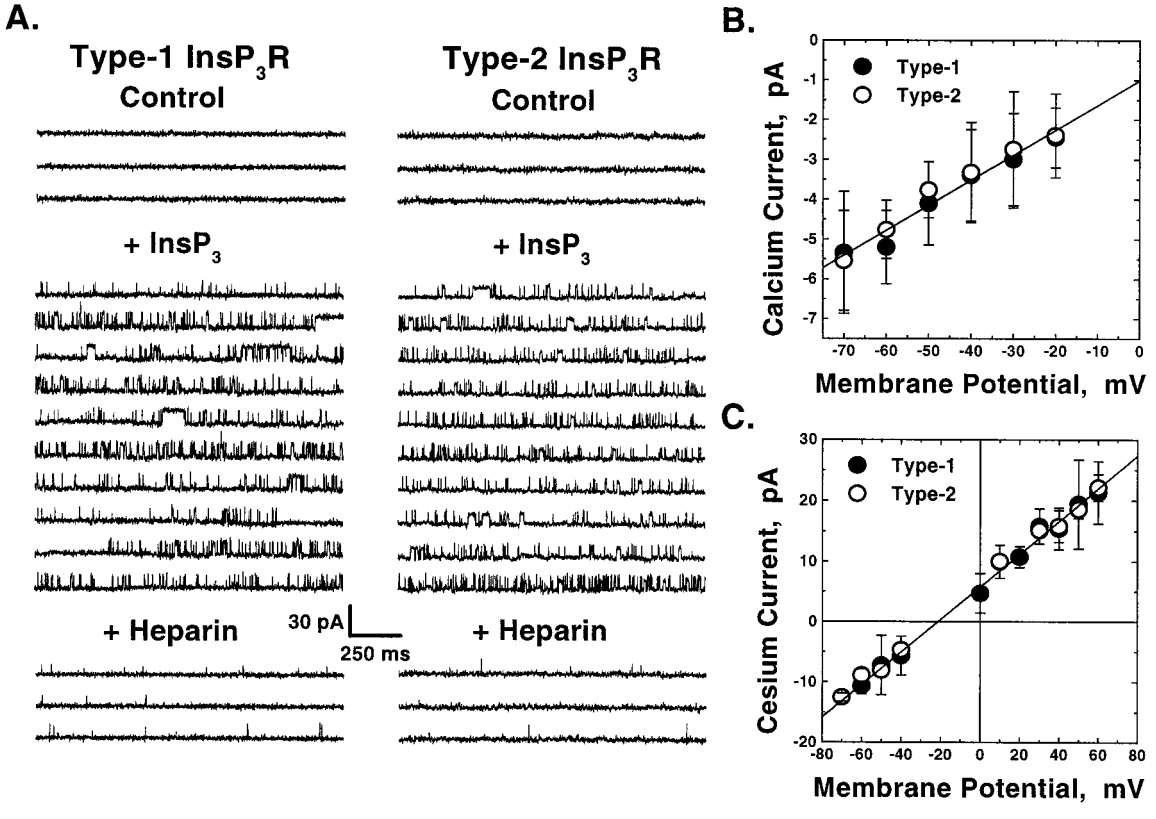


FIGURE 1 Permeation properties of $InsP_3R$ channels. The single-channel properties of type 1 and type 2 $InsP_3R$ channels were defined in planar lipid bilayer studies. Experiments were performed in the presence of 1 $\mu$M $InsP_3$ and 10 $\mu$M ryanodine. (*A*) Sample single-channel records from the type 1 and type 2 $InsP_3R$ channels. Open events (i.e., $Cs^+$ currents) are shown as upward deflections from the zero current level. Control records were recorded before the addition of $InsP_3$ (+$InsP_3$) to the *cis* chamber (1 $\mu$M for type 1; 0.06 $\mu$M for type 2). After several minutes of single-channel recording, heparin (50 $\mu$g/ml) was added to the *cis* chamber (+ Heparin). Solutions contained 220/20 mM $CsCH_3SO_3$ (*cis*/*trans*), 1 mM EGTA, 250 nM free $Ca^{2+}$, 20 mM HEPES (pH 7.4). (*B*) Current-voltage data from the type 1 (●) and type 2 (○) $InsP_3R$ channels conducting $Ca^{2+}$. The two data sets were fit well by the same line (70 pS). Points represent means ± SD ($n > 4$). *Trans* solution contained 50 mM $Ca(CH_3SO_3)_2$ and 20 mM HEPES (pH 7.4). *Cis* solution contained 1 mM EGTA, 250 nM free $Ca^{2+}$, 80 mM HEPES-Tris (pH 7.4). (*C*) Current-voltage data from the type 1 (●) and type 2 (○) $InsP_3R$ channels conducting $Cs^+$. Both data sets were fit well by the same line (280 pS). Points represent means ± SD ($n > 4$). Solutions contained 220/20 mM $CsCH_3SO_3$ (*cis*/*trans*), 1 mM EGTA, 250 nM free $Ca^{2+}$, 20 mM HEPES (pH 7.4).

charge carrier to eliminate $Ca^{2+}$ flux through the pore. This ensures that local $Ca^{2+}$ levels near the channel are precisely controlled. This is important because it has been reported that the $InsP_3$ affinity of the channel may be $Ca^{2+}$ dependent (Yoneshima et al., 1997; Marshall and Taylor, 1994). The cytoplasmic $InsP_3$ sensitivities of both single type 1 and type 2 $InsP_3R$ channels were defined with the free $Ca^{2+}$ concentration buffered at 250 nM on both sides of the channel. Sample single-channel recordings at different $InsP_3$ concentrations are shown in Fig. 2, *A* and *B*. Average open probabilities ($P_o$) are plotted against $InsP_3$ concentration in Fig. 2 *C*. The type 2 $InsP_3R$ channel had higher $InsP_3$ affinity ($EC_{50}$; 58 versus 194 nM for type 1), and the $InsP_3$ dependence of its activation suggests some degree of cooperativity (Hill coefficient; 1.85 versus 0.96 for type 1). The threefold difference in apparent $InsP_3$ affinity is similar to that observed for recombinant ligand-binding domains of the type 1 and type 2 receptors (Südhof et al., 1991; Newton et al., 1994). The dashed line (Fig. 2 *C*) represents previously published type 1 $InsP_3R$ channel data (Watras et al., 1991). A notable difference between the two channels was the extent of $InsP_3$ activation. The open probability ($P_o$) of the type 2 $InsP_3R$ channel was higher than that of the type 1 channel. The extent of this difference will depend on cytoplasmic $Ca^{2+}$ concentration. If the type 1 $InsP_3$ dose dependency data were collected at the optimal cytoplasmic $Ca^{2+}$ concentration (~750 nM), the efficacy difference between the type 1 and type 2 channels would be smaller. Nevertheless, any difference in the extent of $InsP_3$ activation ($P_o$ level) implies that $InsP_3$ efficacy at mobilizing $Ca^{2+}$ is $InsP_3R$ isoform specific. Differential $InsP_3$ efficacy may help explain the high fidelity of $InsP_3$ signaling cascades in cells.

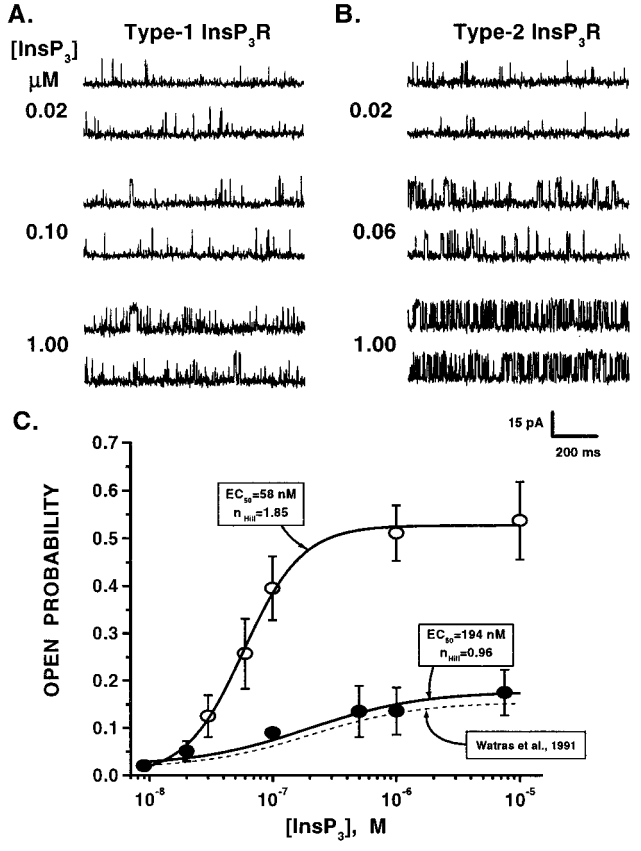


FIGURE 2 The $InsP_3$ sensitivity of single type 1 and type 2 $InsP_3R$ channels defined in planar lipid bilayer studies. Solutions contained 220/20 mM $CsCH_3SO_3$ (*cis*/*trans*), 1 mM EGTA, 250 nM free $Ca^{2+}$, 20 mM HEPES (pH 7.4). All experiments were performed in the presence of 10 $\mu$M ryanodine. The $InsP_3$ concentration was varied in the *cis* chamber. Opening events are shown as upward deflections from the zero current level. Data points are plotted as means ± SD ($n > 6$). (*A*) Sample single-channel records from the type 1 $InsP_3R$ channels at three $InsP_3$ concentrations. (*B*) Sample single-channel records from the type 2 $InsP_3R$ channels at three $InsP_3$ concentrations. (*C*) $InsP_3$ concentration response for single type 1 (●) and type 2 (○) $InsP_3R$ channels. The type 1 $InsP_3R$ channel data from Watras et al. (1991) are represented as a dashed line.

The cytoplasmic $Ca^{2+}$ sensitivity of single type 1 and type 2 $InsP_3R$ channels was defined at a fixed $InsP_3$ concentration (1 $\mu$M). The free $Ca^{2+}$ concentration on the luminal side of the channels was held constant at 250 nM. Sample single-channel records at different cytoplasmic $Ca^{2+}$ concentrations are shown in Fig. 3, *A* and *B*. The average $P_o$ of the type 2 and type 1 channels at various cytoplasmic $Ca^{2+}$ concentrations is plotted in Fig. 3 *C*. The $Ca^{2+}$ dependence of the type 1 $InsP_3R$ channel was sharply bell shaped. Maximum type 1 channel activity occurred near 1 $\mu$M $Ca^{2+}$. These results are similar but not identical to those of Bezprozvanny et al. (1991) (Fig. 3 *C*, *dashed line*). The differences between the two type 1 data sets could be due to the different charge carriers used. Bezprozvanny et al. (1991) used a large luminal $Ca^{2+}$ concentration (~50 mM) to provide the charge-carrying ion. The consequence is that the superphysiological $Ca^{2+}$ flux through the channel may alter the occupancy of cytoplasmic $Ca^{2+}$ and/or impact $InsP_3$ regulation of the channel. In our study, the use of a monovalent charge carrier eliminated this possibility. The two studies also used different methods to isolate single $InsP_3R$ channels. Bezprozvanny et al. (1991) fused native cerebellar microsomes into the bilayer, whereas we fused $InsP_3R$-enriched proteoliposomes.

The $Ca^{2+}$ dependence of the type 2 $InsP_3R$ channel is also illustrated in Fig. 3 *C*. The type 2 $InsP_3R$ channels were active, even at relatively low $Ca^{2+}$ concentrations (~25 nM), compared to the type 1 channels. At higher $Ca^{2+}$ concentrations, type 2 $InsP_3R$ channel activity ($P_o \approx 0.7$) was maintained over a wide $Ca^{2+}$ concentration range. Even at millimolar $Ca^{2+}$ concentrations (data not shown), the $P_o$ of type 2 channel remained high (~40%). Thus the $Ca^{2+}$ dependence of the type 2 channel had essentially a sigmoidal shape, instead of the classical bell shape observed for the type 1 channel. This is interesting because it indicates that the type 2 channel lacks the $Ca^{2+}$ inactivation mechanism that turns off the type 1 channel at micromolar $Ca^{2+}$ concentrations. The type 1 and type 2 $InsP_3Rs$ both encode a cytosolic $Ca^{2+}$-binding site (residues 2124–2146 of the type 1 isoform) that is thought to be involved in the $Ca^{2+}$ regulation of these receptors (Mignery et al., 1992; Sienaert

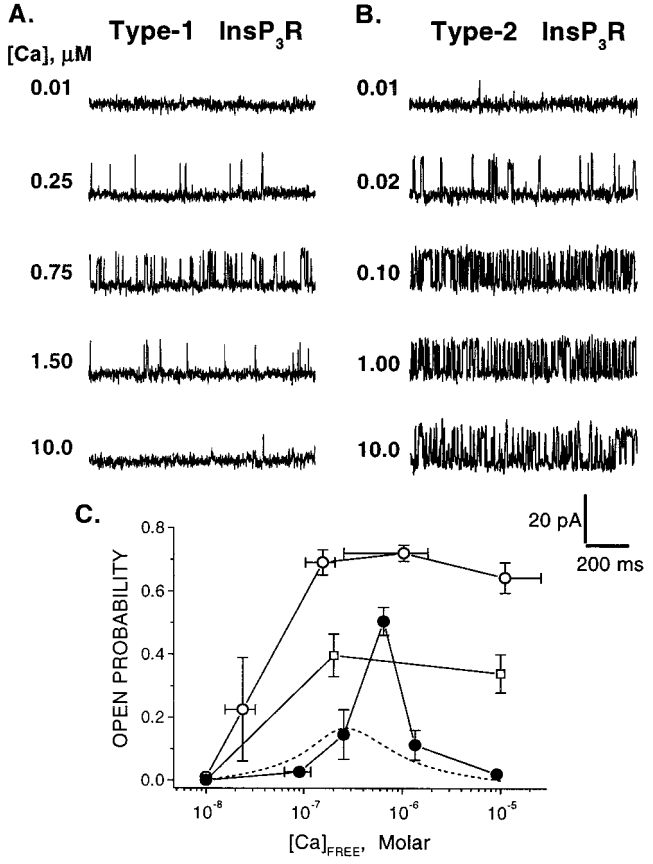


FIGURE 3 The $Ca^{2+}$ sensitivity of single type 1 and type 2 $InsP_3R$ channels defined in planar lipid bilayer studies. Solutions contained 220/20 mM $CsCH_3SO_3$ (*cis*/*trans*), 1 mM EGTA, 250 nM free $Ca^{2+}$, 1 $\mu$M $InsP_3$, 10 $\mu$M ryanodine, and 20 mM HEPES (pH 7.4). The free $Ca^{2+}$ concentration was varied in the *cis* chamber and directly verified by an on-line mini-$Ca^{2+}$ electrode. The free $Ca^{2+}$ concentration in the *trans* chamber was buffered at 250 nM. Opening events are shown as upward deflections from the zero current level. Data points are plotted as means ± SD ($n > 6$). (*A*) Sample single-channel records from the type 1 $InsP_3R$ channels at five $Ca^{2+}$ concentrations. (*B*) Sample single-channel records from the type 2 $InsP_3R$ channels at five $Ca^{2+}$ concentrations. (*C*) The $Ca^{2+}$ concentration response for single type 1 (●) and type 2 (○) $InsP_3R$ channels. Type 1 $InsP_3R$ channel data from Bezprozvanny et al. (1991) are represented by the dashed line. The $Ca^{2+}$ concentration response for single type 2 $InsP_3R$ channels activated by 0.1 $\mu$M $InsP_3$ is also shown (□).

et al., 1996). Therefore, the single-channel data (Fig. 3 C) show that this $Ca^{2+}$-binding region does not contain all of the determinants of $InsP_3R$ $Ca^{2+}$ regulation.

Recently Kaftan et al. (1997) proposed a complex scheme explaining the interaction of $Ca^{2+}$ and $InsP_3$ in the regulation of the type 1 $InsP_3R$ channel. They argue that the inactivation phase of the $Ca^{2+}$ dose dependency of the type 1 channel is masked at high $InsP_3$ concentrations. To support this claim they propose a model that employs a three-dimensional surface to describe the complex interaction between $Ca^{2+}$ and $InsP_3$. In this context, it is possible that the difference between the $Ca^{2+}$ dependencies of the type 1 and type 2 $InsP_3R$ channels may be due to a shift of this surface along the $InsP_3$ scale to higher concentrations for the type 1 and lower for the type 2 channel. To test this possibility, we lowered (10-fold) the fixed $InsP_3$ concentration and reassessed the $Ca^{2+}$ dependency of the type 2 $InsP_3R$ channel (see Fig. 3, *open squares*). The type 2 receptor's response to $Ca^{2+}$ did not become bell shaped, as predicted by the model of Kaftan et al. (1997). Instead, its $Ca^{2+}$ dependency remained sigmoidal, and only its magnitude appeared to be dependent on $InsP_3$ concentration. This result suggests that the interaction of $Ca^{2+}$ and $InsP_3$ in the regulation of type 1 and type 2 channels is different.

## DISCUSSION

We have defined the single-channel function of the type 2 $InsP_3R$ in parallel with the type 1 isoform from cerebellum. The two receptor homologs form channels with very similar permeation properties and unitary conductances. Similar permeation properties are also consistent with the highly conserved nature of the $InsP_3R$ channel domains. These results also suggest that the amplitude of local intracellular $Ca^{2+}$ signals in cells is not likely due to opening of different $InsP_3R$ channels with unique or isoform-specific conductance. It is thus more likely that the heterogeneity in intracellular $Ca^{2+}$ signaling arises from differences in how these channels are regulated.

The regulation of the type 1 and type 2 channels by $InsP_3$ and that by cytosolic [$Ca^{2+}$] were markedly different. $InsP_3$ activated the type 2 channels to a greater extent when the channels were exposed to 250 nM $Ca^{2+}$ (see Fig. 2). The magnitude of this difference will vary, of course, with the cytoplasmic $Ca^{2+}$ concentration. Nevertheless, the open probability ($P_o$) of the type 2 $InsP_3R$ channel was always greater than that of the type 1 channel at saturating $InsP_3$ concentrations. This implies that an $InsP_3$ stimulus will be more effective at mobilizing $Ca^{2+}$ if the type 2 $InsP_3R$ channel isoform is the target. This would allow $InsP_3$ stimuli too small to activate type 1 $InsP_3R$ channels to mobilize $Ca^{2+}$ through type 2 channels. This could in effect segregate parallel $InsP_3$ signaling cascades that use these two different $InsP_3R$ channels.

The type 1 and type 2 $InsP_3R$ channels have clearly different $InsP_3$ binding affinities (Newton et al., 1994; Südhof et al., 1991). The type 2 $InsP_3R$ has the highest $InsP_3$ binding affinity ($K_D \approx 27$ nM) of the three characterized isoforms, with a relative order of type 2 > type 1 ≫ type 3 (Perez et al., 1997; Newton et al., 1994: Südhof et al., 1991). The affinity of the type 1 receptor is ~5–10-fold higher than that of the type 3 receptor. The affinity of the type 2 $InsP_3R$ is approximately threefold higher than that of the type 1 receptor. Interestingly, our data show that the $EC_{50}$ of $InsP_3$ for the type 2 $InsP_3R$ channels was about threefold higher than that of the type 1 channel (58 versus 194 nM). Thus the relative $InsP_3$ affinities measured by $InsP_3$ binding or $InsP_3$ activation of single type 1 and type 2 channels are in good agreement.

Different levels of cooperativity of $InsP_3$ responses in several cellular systems have been reported (for a review

see Mignery and Südhof, 1993). For example, some groups suggest that more than one $InsP_3$ molecule must bind to the $InsP_3R$ channel for it to open (e.g., Meyer et al., 1988; Iino and Endo, 1992). Other groups suggest that $InsP_3$ binding to a single site on the channel complex is sufficient to open the channel (e.g., Watras et al., 1991; Finch et al., 1991). The $InsP_3$ dose dependency of single $InsP_3R$ channel activity in our study indicates that cooperativity of the $InsP_3R$ is isoform specific (see Fig. 2). The activation of the type 2 $InsP_3R$ appeared to involve more than one $InsP_3$ molecule, whereas activation of the type 1 $InsP_3R$ did not. However, the relatively low activity level of the type 1 $InsP_3R$ channel made it difficult to accurately predict its response at low $InsP_3$ levels. Thus our estimation of the $InsP_3$ cooperativity of the type 1 channel should be considered with care. In our view, more single-channel measurements at very low $InsP_3$ concentrations would be required to definitively establish the $InsP_3$ cooperativity of type 1 receptor.

Our results demonstrate that cytosolic $Ca^{2+}$ differentially regulates the two receptor isoforms. Type 2 receptor channel activity was maintained at high $Ca^{2+}$ concentrations ($P_o \approx 0.4$, 1 mM $Ca^{2+}$), whereas the type 1 channels were inactivated at $Ca^{2+}$ concentrations above 1 $\mu$M. The shape of the $Ca^{2+}$ dependence of the type 2 channel was sigmoidal instead of the classical bell shape observed for the type 1 channel. This is an interesting observation because it has implications for the local control of intracellular $Ca^{2+}$ release. For example, the $InsP_3R$ channels mediate relatively large $Ca^{2+}$ release fluxes that must alter the free $Ca^{2+}$ profile in the microenvironment of the channel. The bell-shaped $Ca^{2+}$ depencence of the type 1 channel indicates that $Ca^{2+}$ can feed back and turn the channel off, making the activity of the channel self-limiting. In contrast, the sigmodial $Ca^{2+}$ dependency of the type 2 channel indicates that $Ca^{2+}$ feedback will not have an impact on this channel's function. Termination of type 2 channel activity, therefore, is not mediated by a $Ca^{2+}$-dependent inactivation mechanism. Instead, type 2 channel activity will cease upon depletion of the $Ca^{2+}$ store or removal of the $InsP_3$ signal, and/or through some yet to be identified modulatory protein/factor. The consequence is that the type 1 and type 2 channels may mediate very different types of intracellular $Ca^{2+}$ signals. For example, the type 1 channel could be ideal for mediating small transient $Ca^{2+}$ signals, whereas the type 2 channel could be specialized to mediate large, sustained $Ca^{2+}$ signals.

The action of cytosolic $Ca^{2+}$ on $InsP_3R$ activity has been explored in many different experimental systems. There are reports that the function of $InsP_3R$ is regulated by cytosolic $Ca^{2+}$ in a biphasic manner (e.g., Bezprozvanny et al., 1991; Iino, 1990; Finch et al., 1991). Other studies show that $InsP_3R$ function is not governed by a $Ca^{2+}$-dependent inhibition mechanism and thus does not respond to cytosolic $Ca^{2+}$ in a biphasic manner (e.g., Commbettes and Champeil, 1994; Horne and Meyer, 1995). Here we show that single type 1 channel activity is a biphasic function of cytosolic $Ca^{2+}$ concentration. We also show that type 2 channel activity is a monotonic function of cytosolic $Ca^{2+}$ concentration. Thus the apparent disparity between the previously published results could potentially be explained by isoform specific functional $InsP_3R$ attributes in the different experimental systems used. For example, Bezprozvanny et al. (1991) explored $Ca^{2+}$ regulation of $InsP_3R$ channels isolated from cerebellum, tissue rich in the type 1 $InsP_3R$ protein. Horne and Meyer (1995) explored $Ca^{2+}$ regulation of $InsP_3R$ channels in basophilic leukemia (RBL) cells, a cell line rich in type 2-like $InsP_3R$ proteins (Parys et al., 1995).

In this study, the type 2 $InsP_3R$ channel was isolated from ventricular cardiac myocytes. The specific role of the type 2 $InsP_3R$ channel in the myocyte is unclear. Although the myocyte is clearly specialized to optimize the ryanodine receptor (RyR)-mediated $Ca^{2+}$ signaling events that govern cardiac contractility, it must undergo the routine cellular $Ca^{2+}$ signaling that sustains and modulates numerous metabolic and developmental events in cells. Interestingly, the sigmoidal $Ca^{2+}$ dependence of the type 2 $InsP_3R$ channel makes it largely unaffected by the large, repeated, RyR-mediated $Ca^{2+}$ signals. This would effectively limit the cross-talk between RyR-mediated and type 2 $InsP_3R$-mediated $Ca^{2+}$ signals. Thus the sigmoidal $Ca^{2+}$ dependence of the type 2 $InsP_3R$ channel may allow $InsP_3$-dependent intracellular signaling cascades in the myocyte to operate independently of the cardiac contractile cycle. The identity, localization, and role of these $InsP_3$ dependent signaling cascades remain to be determined.

In summary, the resting $Ca^{2+}$ concentration in most cells is $\sim$100 nM and would rarely (if ever) exceed 1 mM, even in small, localized regions. At these free $Ca^{2+}$ concentrations (100 nM to 1 mM), type 2 $InsP_3R$ channels will be active in the presence of $InsP_3$. In contrast, the type 1 channel would not be active when cytosolic $Ca^{2+}$ reaches the micromolar level. Furthermore, $InsP_3$ is more effective at mobilizing $Ca^{2+}$ through the type 2 $InsP_3R$ channel. These data suggest that the type 1 and type 2 channels mediate different types of intracellular $Ca^{2+}$ signals. Type 1 $InsP_3R$-mediated signals would be self-limiting, as $Ca^{2+}$ can feed back and turn off the channel. Type 2 $InsP_3R$ signals would be larger (because of greater $InsP_3$ efficacy at mobilizing $Ca^{2+}$) and would simply follow local $InsP_3$ levels, regardless of local $Ca^{2+}$ concentration. Because most cells contain multiple types of $InsP_3Rs$ (Newton et al., 1994; De Smedt et al., 1997), complex patterns of local $Ca^{2+}$ signaling can arise. Thus it is very likely that isotype-specific functional heterogeneity contributes to the spatial and temporal complexity of intracellular $Ca^{2+}$ signaling in mammalian cells.

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