Commentary

Subtype-specific Regulation of Inositol 1,4,5-Trisphosphate Receptors: Controlling Calcium Signals in Time and Space

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A rise in intracellular calcium ($[Ca^{2+}]_i$) in response to binding of inositol 1,4,5 trisphosphate ($InsP_3$) to its receptors is a ubiquitous signaling system, which is implicated in the control of a myriad of cellular functions (Berridge, 1993). Three distinct $InsP_3$ receptors are expressed to varying degrees in individual cell types (Wojcikiewicz, 1995). Activation of these receptors results in $[Ca^{2+}]_i$ signals that display impressive fidelity, frequently manifested through a complex array of temporal and spatial characteristics. $[Ca^{2+}]_i$ signals in nonexcitable cells are initiated in defined regions (Kasai and Augustine 1990; Rooney et al., 1990); can be spatially limited to a portion of a cell (Kasai et al., 1993) or alternatively spread as repetitive, global, oscillatory waves throughout an entire cell (Lechleiter et al., 1991; Straub et al., 2000). In addition, the periodicity of these spatial events is tightly regulated and has been proposed to provide effectors with a digitally encoded signal.

It has been tempting to speculate that many characteristics of these specific $[Ca^{2+}]_i$ signals are due to the differential regulation of individual $InsP_3$ receptor subtypes expressed in a given cell. However, this idea has been difficult to test because individual receptor types are rarely found in isolation, without "contamination" from other receptor types. Two papers in this issue of *The Journal* have elegantly addressed the issue of subtype-specific regulation of $InsP_3$ receptors by perhaps its most important regulator, calcium. By patch clamping the outer nuclear membrane of isolated *Xenopus laevis* nuclei, Mak et al. (2001 a,b) studied the activity of the type-3 receptor and its regulation by Ca^{2+} and $InsP_3$. Using an "on nucleus" patch-clamp configuration analogous to the "on cell" configuration conventionally used in intact cells, the authors investigated the effects of $InsP_3$, Ca^{2+}, and ATP on $InsP_3$ receptor single-channel activity. Because the outer nuclear membrane is contiguous with the endoplasmic reticulum (ER), $InsP_3$ receptor channel activity can be assessed in an essentially native membrane environment. The authors transfected type-3 $InsP_3$ receptor into oocytes expressing extremely low levels of endogenous (type-1) receptor such that the activity of type-3 receptor could be assessed on an essentially null background, and then subsequently compared with the properties of the type-1 receptor. Previous studies have showed that different $InsP_3$ receptor types display remarkably similar properties. This study and others have shown that in terms of gating, the two channels have an absolute requirement for $InsP_3$ and, after binding of $InsP_3$, exhibit essentially identical maximal open probabilities and display similar conductance properties (Bezprozvanny and Ehrlich, 1994; Mak and Foskett, 1997; Ramos-Franco et al., 1998; Patel et al., 1999; Hagar and Ehrlich, 2000; Mak et al., 2000, 2001 a,b). In oocyte nuclei, both channels are regulated biphasically by calcium, and this modulation is steeply dependent on $[InsP_3]$. Similar conclusions were reported in two recent studies (Swatton et al., 1999; Boehning and Joseph, 2000), but this finding has attracted controversy because the original report of type-3 receptor modulation by Ca^{2+} did not demonstrate inhibition at high $[Ca^{2+}]$ (Hagar et al., 1998). No clear explanation for these disparate results is immediately obvious, but differences in the experimental system (bilayers derived from mammalian cells versus in situ patching of amphibian nuclei) may help to rationalize the discrepancy. Mak et al. (2001a) report that at low $[InsP_3]$ (<20 nM), type-3 receptors are inhibited at Ca^{2+} levels marginally elevated above basal $[Ca^{2+}]_i$, whereas at saturating $[InsP_3]$, inhibition of channel activity does not occur until Ca^{2+} levels are >10 μM.

Of particular note, the major defining difference between the type-1 and type-3 receptors reported by Mak et al. (2001a) is the sensitivity of the receptors to activation by Ca^{2+}. The type-1 receptor has a half-maximal activating $[Ca^{2+}]$ of 210 nM and the activation exhibits cooperativity, the type-3 receptor is half-maximally activated at 77 nM with a Hill coefficient of 1. Thus, the type-3 receptor exhibits robust channel activity at $[Ca^{2+}]$ near resting values, much below the levels where the type-1 receptor is active (Hagar and Ehrlich, 2000). In contrast to inhibitory modulation, the enhancement of channel activity by Ca^{2+} under these conditions showed no dependence on $[InsP_3]$. Thus, for both channels, the predominant effect of Ca^{2+} was modulation of the closed state dwell-time distribution, rather than the time the channel spent in the open

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state. This led the authors to propose a common mode of $InsP_3$ receptor gating, whereby $InsP_3$ relieves Ca^{2+} inhibition by modulating the stability of the inhibitory Ca^{2+} binding site (Mak et al., 1998, 2001a).

Mak et al., 2001b report a critical role for ATP in conferring the absolute and, thus, differential sensitivity of the two receptor types to Ca^{2+}. At zero ATP and saturating [$InsP_3$], both receptors are reported to have identical Ca^{2+} sensitivity. As the [ATP] is increased, the Ca^{2+} concentration for half-maximal activation of each receptor decreases markedly. This effect occurred disproportionately, such that at levels of ATP thought to be close to physiological (≈0.5 mM) the type-3 receptor, when compared to the type-1 receptor, now exhibited increased sensitivity to activation by Ca^{2+} and no cooperativity.

The differential sensitivity of the two channel types to Ca^{2+} and ATP has obvious implications for the role these channels play in the repertoire of calcium signals observed in nonexcitable cells. Resting $[Ca^{2+}]_i$ is generally reported to be in the range between 30 and 150 nM, such that the [Ca^{2+}] for half-maximal activation of the type-1 and type-3 receptors is poised on either side of the basal $[Ca^{2+}]_i$, hinting at distinct functional roles for the two receptors. For example, the Ca^{2+} sensitivity and lack of cooperativity of the type-3 receptor results in high open probability upon binding of $InsP_3$ at or near resting levels of Ca^{2+}. Therefore, the type-3 receptor, is ideally suited to act as an initial trigger for Ca^{2+} release, as originally suggested by Ehrlich and colleagues (Hagar et al., 1998). This is of particular interest in many polarized cell types where $[Ca^{2+}]_i$ signals initiate focally in distinct subcellular domains (Rooney et al., 1990; Kasai and Augustine, 1990; Kasai et al., 1993; Thorn et al., 1993; Straub et al., 2000). Probably the best described system is the pancreatic acinar cell. In this classic system, $[Ca^{2+}]_i$ signals originate in the extreme apical portion of the cell, a region where the localization of each $InsP_3$ receptor type overlaps (Nathanson et al., 1994; Lee et al., 1997; Yule et al., 1997). Although, $InsP_3$-binding data would predict that the type-2 receptor would provide the initial trigger (Sudhof et al., 1991; Newton et al., 1994; but see Nerou et al., 2001), the data reported by Mak et al. (2001a) suggest that the type-3 receptor, because of its functional efficacy at basal $[Ca^{2+}]_i$, is in fact a major player in this initial Ca^{2+} release. Confirmation of the type-3 receptor as the primary trigger will have to wait until a direct functional comparison of the Ca^{2+} activation of type-2 and -3 receptor is made. Notwithstanding, this idea is consistent with a recent study in corneal epithelial cells, which demonstrated that although type-1 receptors were localized close to the site of agonist stimulation at the basal membrane, the Ca^{2+} signal was triggered at a distal site (close to the apical membrane) where type-3 receptors were expressed (Hirata et al., 1999).

At threshold agonist concentrations, where very low $InsP_3$ levels are likely to be generated, $[Ca^{2+}]_i$ signals often are confined to a region close to the initiation sites, even without a contribution of active buffering systems such as mitochondria (Straub et al., 2000). Mak et al. (2001a) provides insight into the mechanisms contributing to this phenomenon. Once released, Ca^{2+} has the potential to interact with neighboring type-1 and -3 receptors. But $[Ca^{2+}]_i$ above 200 nM will inhibit further calcium-induced calcium release (CICR). Therefore, Mak et al.'s results lead to the prediction that even moderate increases in Ca^{2+} above basal levels would inhibit further release by type-3 receptors, and especially by type-1 receptors, which are more sensitive to Ca^{2+} inhibition at low [$InsP_3$] (Mak et al., 1998). This inhibition, evident at very low $InsP_3$ levels, is consistent with an observation reported by Taylor and colleagues that only receptors not bound with $InsP_3$ are refractory because the inhibitory Ca^{2+} binding site on the $InsP_3$ receptor is accessible only when the receptor is not bound by $InsP_3$ (Adkins and Taylor, 1999). Thus, at threshold [$InsP_3$], Ca^{2+} released from the trigger pool of receptors would be more likely to interact with receptors not bound by $InsP_3$. The implication is that highly localized sites of release may be subject to augmentation by CICR but that "lateral inhibition" of receptors would occur, effectively limiting the initial signal (Adkins and Taylor, 1999). Indeed examples of substantial (≈1 μM), highly localized elevations of $[Ca^{2+}]_i$ have been reported upon threshold stimulation (Ito et al., 1997).

Although the type-3 receptor exhibits properties that make it ideally suited for the initial trigger, it may not satisfy the requirements for regenerative release. For example, a further increase in Ca^{2+} will increase channel open probability less than threefold and, thus, the dynamic range of CICR is limited (Mak et al., 2001a). In contrast, when $InsP_3$ levels are significantly increased, elevated Ca^{2+} will facilitate robust CICR from type-1 receptors because the open probability of the channel in response to Ca^{2+} varies 20-fold, the increase in channel activity is cooperative, and fine grading of CICR over a wide range of $[Ca^{2+}]_i$ is possible (Mak et al., 2001a). Thus, CICR through type-1 receptors can provide the fundamental events required to transmit signals in the form of propagating Ca^{2+} waves.

The modulation of $InsP_3$ receptors by ATP over a physiologically relevant range is particularly noteworthy (Mak et al., 2001b). This provides the cell with a mechanism to establish a "set-point" for $InsP_3$ sensitivity when the concentration of ATP is dynamically regulated. This property could be important under conditions where cells are metabolically compromised or have a particularly active metabolism. Perhaps more importantly, $InsP_3$ receptors localized to the ER where SERCA pumps are abundant, or to regions that are close to mi-

tochondria, could be subject to dynamic regulation as ATP levels change in the surrounding microdomain (Kennedy et al., 1999). For example, a positive feedback mechanism could be initiated as the Ca^{2+} released from the ER is sequestered by mitochondria, leading to the activation of dehydrogenases vital to the production of ATP. The released ATP could then further increase the sensitivity of $InsP_3$ receptors to activation by Ca^{2+}. Of course, a fine balance exists because elevated Ca^{2+} may lead to the increased activity of Ca^{2+}-ATPases and the subsequent consumption of ATP. This scenario does provide a sobering point of caution reflecting the state of the field: although not detracting from this study, these data are obtained at steady-state levels of $InsP_3$, Ca^{2+}, and ATP. Under physiological conditions, the situation is much more complicated because all three of these parameters are changing and further subject to regulation by other important inputs such as phosphorylation (Giovannucci et al., 2000) and modulation by accessory proteins including calmodulin and FK506 binding proteins (Patel et al., 1999). Nevertheless, the results of the Mak et al. (2001a,b) reports provide important fundamental insights into how the differential regulation of $InsP_3$ receptors by calcium may dictate which receptor types are likely to contribute to the initiation, localization, and propagation of $[Ca^{2+}]_i$ signals in cells which express multiple $InsP_3$ receptor types.

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