

Signaling Microdomains: InsP₃ Receptor Localization Takes on New Meaning

A fundamental question in cell biology is how different receptor-mediated signaling cascades, despite utilizing many of the same intracellular components, can generate specific cellular responses. Delmas and colleagues (in this issue of *Neuron*) address this question in relation to the muscarinic acetylcholine receptor (M₁AchR) and the B₂ bradykinin receptor (B₂R). Using Trp channel isoforms as biosensors for PLC stimulation in response to agonist activation, they demonstrate a role for signaling microdomains in the induction of such selective responses.

Differences in the spatial, temporal, and quantitative aspects of intracellular calcium signals encode a wide array of neuronal functions (Berridge, 1998). In a paper in this issue of *Neuron*, Delmas and colleagues provide strong support for a new concept in neuronal calcium signaling (Delmas et al., 2002). Functional evidence is described showing that the input specificity and sensitivity of InsP₃-mediated calcium signaling is determined by spatial proximity of G protein-coupled receptors and InsP₃ receptors in signaling microdomains. Previous reports had shown that in sympathetic ganglion cells, bradykinin-activated B₂ receptors deactivate the inhibitory M current via InsP₃-induced calcium release, whereas muscarinic M₁ receptors use an alternative, yet undefined, pathway that is relatively inefficient at releasing calcium via the second messenger InsP₃ (Cruzblanca et al., 1998). However, since both receptors are coupled to the same type of *pertussis* toxin-insensitive Gq protein, the question arises as to what mechanisms mediate the specificity of these signaling interactions.

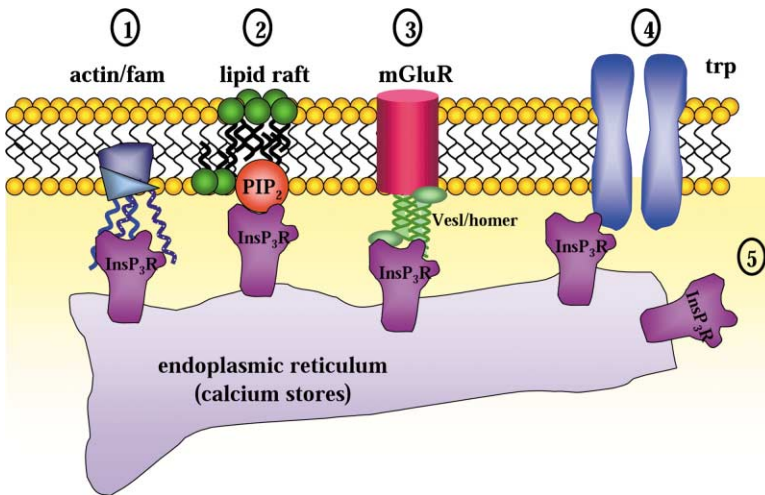
Based on this stunning paradox, the authors established an elegant assay system enabling them to distinguish between two signaling pathways initiated by PLC β cleavage of PIP₂, the production of diacylglycerol (DAG), and calcium release induced by InsP₃. By expressing different transient receptor potential (trp) channels that are either selectively activated by DAG (mTrpC6) or connected to InsP₃-mediated calcium signaling (hTrpC1), they were able to measure currents that identified the second messenger system(s) activated by muscarinic or bradykinin receptor agonists with a high specificity. Apart from being a great tool to dissect those two main components of PLC β -mediated signaling, this method indirectly mirrors the real time course of DAG production and InsP₃-induced calcium release, allowing a view into the *in vivo* activity of PLC β . These dynamic measurements of second messenger generation confirm that both bradykinin and the muscarinic agonist Oxo-M generate DAG to activate mTrpC6. In contrast, bradykinin activation of B₂ receptors leads to activation of InsP₃-

induced calcium release, whereas the muscarinic agonist Oxo-M does not. Comparison of the kinetics of the currents evoked by DAG activation of mTrpC6 when stimulating cells with bradykinin and Oxo-M clearly shows that both agonists are equally efficient at activating PLC β .

With this ability to functionally separate the signaling pathways of the two receptors, it was then possible to characterize the mechanisms underlying the higher efficiency of one pathway over another. Biochemical and morphological evidence were provided to show that the spatial proximity of B₂ receptors and InsP₃ receptors form “signaling microdomains” in the subplasmalemmal space. In contrast, M₁ receptors are randomly distributed and do not seem to be directly linked to the InsP₃ receptors. It is this distinctive mechanism that renders InsP₃ receptors more sensitive to bradykinin than to muscarinic stimulation.

These findings lead directly to the question of how the proximity between InsP₃ receptors and plasmalemmal G-proteins is established and how specificity of this interaction is generated for distinct types of G proteins. One can imagine a number of ways that the cell could accomplish this goal (see Figure). The authors identify the actin cytoskeleton (Figure, #1) to be of crucial importance for mediating the spatial proximity of InsP₃ receptors, B₂ receptors, and PLC β . An additional factor that could establish this close connection is a tight interaction between InsP₃ receptors and plasmalemmal PIP₂ (Lupu et al., 1998). This direct coupling between the InsP₃ receptor and its lipid precursor (Figure, #2) could mediate the proximity of G protein-coupled receptors in the plasma membrane and the InsP₃ receptors, especially if it is assumed that lipid rafts of increased PIP₂ content are clustered around specific GPCRs. Cofactors may also be identified that would mediate the specificity of this type of signaling microdomain formation (Figure, #3). In this context, the clustering of metabotropic glutamate receptors (mGluRs) and InsP₃ receptors was shown to be determined by the homer family of proteins (Tu et al., 1998). However, there is no homer binding motif in bradykinin B₂ receptors. It is tempting to speculate that a number of families of scaffolding proteins mediate specific interactions between G protein-coupled receptors and InsP₃ receptors that could then modulate the specificity of receptor-mediated InsP₃ pathways in different cells depending on the expression pattern of each of the signaling pathway components. Alternatively, there can be direct coupling between the intracellular receptor and its plasma membrane partner (Figure, #4). Indeed, a link between the InsP₃ receptor and trp was shown both functionally and biochemically (Kiselyov et al., 1998).

Another important aspect of their functional description of signaling microdomains is the relationship between the domains and the spatial and temporal patterning of the calcium signal. This is closely related to the observation that there are reproducible signal initiation sites at proximal dendrites for neuronal calcium waves initiated by different G protein coupled receptor ago-



InsP₃ Receptor (InsP₃R) Location in the Endoplasmic Reticular Membrane Can Be Modulated by Interactions with Specific Regions of the Plasma Membrane

(1) Actin and focal adhesion molecules (fam) can enmesh the InsP₃R. (2) Binding between the InsP₃R and PIP₂ found in lipid rafts can facilitate rapid signaling. (3) Scaffolding proteins such as Vesl/homer can link the InsP₃R to specific receptors. (4) InsP₃R can bind directly to plasma membrane receptors such as trp. (5) InsP₃R can remain unbound in the endoplasmic reticular membrane.

nists (Nakamura et al., 2000). The principle underlying the generation of these waves is based on activation of clusters of InsP₃ receptors and ryanodine receptors that produces elementary calcium release events. Signal initiation is achieved by enhancing the frequency and coupling of elementary calcium release events according to stimulus strength (Koizumi et al., 1999). With the ability to separate specific agonist responses (Delmas et al., 2002) and with a better temporal and spatial resolution when measuring intracellular calcium signals, it will be possible to analyze the calcium dynamics of signal initiation in the described signaling microdomains and to identify them as pacemakers for global calcium transients. It is important to note that InsP₃ receptors and ryanodine receptors generally act together where the release of calcium from the InsP₃ receptors leads to activation of ryanodine receptors following activation of G proteins. The consequences of this cascade can be spatially specific (Johanning et al., 2002) where both the magnitude and frequency of the response can be modulated, and the inclusion of multiple components into the signaling microdomain adds a whole new layer of complexity to signal formation.

Another direction that emerges from the findings of Delmas and colleagues is to determine the factor(s) responsible for the increased sensitivity of InsP₃ receptors in signaling microdomains. If we consider the fast diffusion rate of InsP₃ and its resulting role as a global second messenger and the even distribution of B₂ receptors all over the cell body (see Figure 9 in Delmas et al., this issue), it is difficult to imagine that focal gradients of InsP₃ develop that could explain the higher sensitivity of InsP₃ receptors in signaling microdomains. A kinetic argument assuming a faster rate of rise of the InsP₃ concentration in the vicinity of its production site resulting in a more efficient activation of InsP₃Rs could be an explanation. However, more data on InsP₃ receptor rapid kinetics and subcellular differences in InsP₃ generation are needed to support this hypothesis. Delmas and colleagues (2002) propose a mechanism that is related to the inhibitory effect of calmodulin on InsP₃ receptor function (Michikawa et al., 1999). When a non-calcium-binding mutant of calmodulin was overex-

pressed in sympathetic ganglion cells, it was possible to reconstitute muscarinic receptor activation of InsP₃-induced calcium release. From these results, they suggest a model in which calmodulin serves as a filter that is more efficient in inhibiting slow rather than rapid rises in InsP₃. In this way, the authors suggest that the kinetics of the InsP₃-mediated calcium response become a major factor in determining the regenerative spread of a calcium wave. Again, more data are needed to support this hypothesis. It is also possible that associated proteins have a direct effect on InsP₃ receptor function, either enhancing or inhibiting calcium release. For example, in neuronally differentiated PC12 cells, signal initiation occurs in the neurite and this is determined by factors downstream of InsP₃ production. Although the differential sensitivity may be determined by the distribution of biophysically distinct InsP₃ receptor isoforms (Thrower et al., 2001), this explanation is not sufficient for many other cell types. Another explanation suggested for the experiments using PC12 cells is the complementary distribution of chromogranin (Johanning et al., 2002), a protein found in the lumen of the ER that has profound effects on InsP₃ receptor function (Thrower et al., 2002). However, the number of possible candidates that could subsume the role of regulatory cofactor is nearly limitless.

In sum, determining who the partners are and the proximity, specificity, and sensitivity in signaling microdomains is going to be an exciting focus of future research in neuronal calcium signaling.

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Have We Been Hebbing Down the Wrong Path?

In this issue of *Neuron*, Stepanyants, Hof, and Chklovskii derive a simple mathematical formula to calculate, from measurable anatomical parameters, the capacity for neuronal wiring plasticity involving local synaptic rearrangements. Their work provides a deeper understanding of the potential contribution of structural plasticity to learning and memory.

“When in the course of synaptic events, it becomes necessary for one neuron to dissolve (or potentiate) the bonds which have connected it to another, a decent respect to the opinions of neurophysiologists requires that they should declare the causes which impel them to the separation (or potentiation).”

Pull the average neuroscientist off the street and ask them how learning occurs in the brain, and you’re likely to get a reflex response that includes such pat phrases as “activity-dependent changes in synaptic strength,” “LTP/LTD,” or “Hebbian learning.” The Hebb doctrine, which basically says that “neurons that fire together wire together,” emphasizes the view that dendrites are passive collectors of their synaptic input, that the connection between neuron A and neuron B can be represented by a single number (positive or negative), and that that number can change as an outcome of learning. This has arguably been the most influential idea about the neural substrate for learning in the history of the field. But is it right?

When the brain is first wired up during development, the physical interface between axons and dendrites is extremely dynamic, involving large-scale growth, retraction, and remodeling of axonal and dendritic arbors on timescales of minutes to hours (Cline, 1999). In the adult nervous system, the physical remodeling of axons and

dendrites continues, evidently throughout life (Woolley et al., 1990; Darian-Smith and Gilbert, 1994; Klintsova and Greenough, 1999).

Given that dendrites are anatomically and physiologically complex and are the principal substrates for information processing within the neuron itself (Stuart et al., 1999), the question arises as to the consequences, if any, of axo-dendritic structural plasticity for learning and memory. In other words, does experience-dependent remodeling of the interface between axons and dendrites have any special role in the long-term storage of information in the brain, beyond that associated with the establishment and fine tuning of the brain’s basic point-to-point wiring diagram? Could there be lurking here some form of structural neural plasticity that falls outside the scope of the conventional Hebb doctrine, that is, that cannot be described in terms of changes in the overall connection strength between the pre- and postsynaptic neurons?

In this issue of *Neuron*, Stepanyants, Hof, and Chklovskii (Stepanyants et al., 2002) derive a simple mathematical formula that quantifies one very basic form of structural plasticity in the neural wiring diagram, that is, changes to the neural circuit that require only incremental physical adjustments to the mesh of interdigitated axons and dendrites. Most synaptic contacts are formed on dendritic spines, small protrusions from the dendritic shaft up to a few microns in length. Stepanyants and colleagues use elegant but straightforward geometric arguments to calculate how many different axons course within a spine’s reach of any given dendrite, since these axons could in principle make or break synaptic connections with their dendritic partners by simply adding or deleting spines between them. One reason the authors focus on spines has to do with the timescale of learning: spine changes can occur on a timescale of minutes (O’Rourke and Fraser, 1990; Dailey and Smith, 1996; Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Toni et al., 1999; Lendvai et al., 2000) and could thus mediate relatively fast forms of learning. In the densely packed adult neuropil, more extensive structural remodeling does occur, but on much longer timescales (Greenough and Bailey, 1988; Darian-Smith and Gilbert, 1994).

As it happens, Stepanyants et al. show that with relatively few assumptions, the number of axons accessible to a dendrite can be expressed as the product of the dendritic cross section, the average interbouton distance, and the synapse density. They then use their expression to calculate the “filling fraction” for various brain areas. What is the filling fraction? It is the probability that a synapse is formed if an axon is within a spine’s reach of a dendrite. If the filling fraction is 0.2, that means that 20% of the candidate axons have been “chosen” by the learning rule to actually form synaptic contacts with a given dendrite. But which 20%? Having the flexibility to choose is key (Poirazi and Mel, 2000).

Assuming there are at least as many nearby axons as there are dendritic sites to fill, the filling fraction ranges from 0 to 1. But which is better from the point of view of memory capacity, a low or a high filling fraction? It turns out the correct answer is “medium.” Consider a grocery store that sells 480 different food items (presyn-