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Message Delivered: How myocytes control cAMP signaling

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Despite the vast array of specific receptors and ligands through which cells receive information from their outside environment, the first line of messengers that relay this onto internal signaling cascades is more limited. Yet somehow, the purpose of the original message is conveyed (i.e. we know who knocked on the front door) and the message ends up influencing just the right proteins to produce the desired effect. A striking example of this selectivity is the cyclic nucleotides for which only two basic types exist; cAMP and cGMP. Despite this limited cast, these molecules precisely regulate many complex and disparate cellular activities. Their trick is to be in the right place at the right time and with the right amount of signal. It is now well established that cyclic nucleotides are spatially compartmentalized to enable different external triggers to vary the internal cellular response[1–5]. This can be achieved by localizing the cyclases that synthesize cAMP or cGMP in different parts of the cell, parking the effector kinase PKA in particular regions by means of anchoring proteins (AKAPs)[6] (may apply to PKG though still unclear), and targeting phosphodiesterases to one area or another and/or providing them with hierarchical control[1]. The latter is thought to be particularly important by limiting diffusion of a cyclic nucleotide from its site of generation to other parts of the cell. There are 11 PDE subfamily members with about 50 isoforms, targeting cAMP, cGMP, or in some instances both[7]. Given that many of these PDEs can themselves be activated by cyclic nucleotides and/or their effector kinases, the system has substantial built-in complexity for localized regulation. Compartmental signaling was first reported and remains best characterized for cAMP[2,4–6,8] and even PKA[9] itself. Compartmentation was more recently revealed for cGMP as well[3,10,11], though imaging tools useful in myocytes remain under development[12,13] and mechanisms are less well understood. The primary tools used to examine compartmentation of cyclic nucleotide signaling have been reporters such as olfactory cyclic nucleotide-stimulated ion channels that when expressed reside in the outer sarcolemmal membrane, and probes based mostly on fluorescent energy transfer to reveal the presence of a cyclic nucleotide. FRET-based studies in neonatal myocytes first revealed that cAMP stimulated by beta-adrenergic agonists appears in micro-domains near T-tubules/junctional SR, and its diffusion is markedly limited by localized PDEs[2]. This was further examined in adult myocytes using a mouse expressing a cAMP-sensitive FRET probe in these cells, which showed differential stimulation of cAMP pools that depended upon whether β1 or β2 receptors were targeted[4]. The β2 pool remained at the outer membrane while the former

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started there but quickly diffused broadly into the cytosol. In another study using an outer membrane cAMP-reporter ion channel, β1-stimulated cAMP was detected whereas β2-stimulated cAMP was not. The latter was greatly enhanced if both PDE3 and PDE4 (cAMP esterases) were co-inhibited, while for β-1, inhibition of either PDE enhanced the cAMP signal[14]. Both studies found that between the two PDEs, PDE4 was more potent with respect to cAMP regulation. Importantly, these and essentially all prior studies examined sustained (tonic) stimulation responses and used a single probe (membrane channel or FRET) to detect spatial cAMP changes. They did not address the more rapid kinetics of the signaling and differential role of PDEs in such kinetics, features that could contribute to compartmentalization. They also did not accurately detect cAMP kinetics at different sites simultaneously.

In the current issue of *Circulation Research*, Leroy and colleagues[15] start to fill in this gap – providing the first analysis of cardiac myocyte cAMP regulation kinetics simultaneously using an outer membrane channel sensor and cytosolic FRET probe. Importantly, both have rapid responses, the FRET probe being based on an EPAC cAMP binding sequence. Following short exposure to the β-AR agonist isoproterenol, cAMP rose first at the outer membrane and slightly later in the cytosol, with the latter also showing slower decay kinetics. As a physiologic read-out, the investigators assessed L-type Ca^{2+} current density showing this to peak after maximal cAMP, and declined with a much longer time constant (well after cAMP levels had fallen to baseline).

The different kinetics suggested regulatory PDEs might be involved. To test this, selective PDE4 and PDE3 inhibitors were administered. PDE4 inhibition markedly increased the peak cAMP and Ca^{2+} current response, and also prolonged the decay of the transient – particularly in the FRET-detected cytosolic cAMP signal. In striking contrast, PDE3 inhibition only slightly increased the peak cAMP at the membrane, and had no detectable influence on the cytosolic cAMP response nor on Ca^{2+} current. However, when both PDEs were inhibited, the residual decay of both cytosolic cAMP and Ca^{2+} current was effectively prevented, and membrane localized cAMP decay markedly slowed. This indicates that PDE3 could regulate cAMP but only if PDE4 was already inhibited, supporting hierarchical control that could relate to differential activation and/or localization.

One way PDEs are activated is by phosphorylation by A- and/or G-kinases; PKA in the case of PDE4 and PDE3. This feedback mechanism means that a rise in cAMP stimulates the relevant PDE (via PKA) to reduce the initiating signal. PKA appeared to particularly activate PDE4 in their study, having a smaller impact on PDE3. Indeed, inhibition of PDE4 with or without concomitant inhibition of PKA resulted in a similar cytosolic cAMP transient response, indicating that the primary PKA moderating target was PDE4. PKA activation is also presumed to explain the more prolonged I_{Ca} decay, though this was not directly examined. It may also explain why PDE3 inhibition influenced cAMP hydrolysis only after PDE4 was first inhibited. Inhibiting PDE4 would then be expected to further elevate PKA activity, enhancing PDE3 regulation. This would indicate yet another mechanism for selective regulation – hierarchical control based on relative catalytic activities controlled by feedback loops.

The findings in Leroy et al[15] should be considered in the context of the specific experimental design and models used. For example, the dominance of PDE4 over PDE3 regulation may relate to the use of rat myocytes, which unlike dog, rabbit, or human, have far less PDE3 and more PDE4 regulating their cAMP hydrolysis[16]. Mouse is similar to rat in this regard. In isolated human and rabbit myocytes, I_{Ca} amplitude increased with a PDE3but not PDE4 inhibitor[17]. PDE3 inhibition has been widely tested in humans as a positive inotropic agent[18] – both by itself and with concomitant β-AR, where it was shown to

augment contractility[19]. Thus, whether the same cAMP kinetics and differential regulation by the two PDEs applies to other mammalian species remains unknown. Another issue relates to the short time-constant of ISO exposure. In a prior study, the investigators found PDE3 inhibition could enhance outer membrane cAMP in rat adult myocytes when exposed to sustained β1 stimulation, albeit still less so than with PDE4 inhibition. Furthermore, both PDE3 and PDE4 inhibition augmented Ca^{2+} transients,SR Ca^{2+} content, and phospholamban phosphorylation in mice, with PDE4 modulation shown to PI3Kγ-dependent[20]. Thus, the relative role of the two PDEs may also depend upon the chronicity of the stimuli that may further recruit feedback loops yet identified. Both PDE3 and PDE4 are expressed as multiple genes and isoforms, and certainly in the case of PDE4, this results in specific to sub-cellular targeting (i.e. PDE4D3 with AKAP18 at the SR, PDE4D5 with β-arrestin, and PDE4D4 interacting with SH3-domains on tyrosine kinases such as Fyn and Src)[21]. The commonly used PDE4 inhibitor rolopram (used in the current study) does not confer isoform selectivity, so even more sub-compartmentalized signaling is likely. In addition to these two PDEs, PDE2 has been shown to regulate a pool of cAMP coupled with NOS activation[5], and PDE8[7], also a cAMP-PDE, may also contribute in a manner that remains to be elucidated.

In the science of cyclic nucleotides and PDEs, compartmentalization is becoming a mantra, helping explain complex signaling behavior and targeting of the message. Much remains to be discovered, including whether disease disrupts localized targeting so a message delivered can get garbled. Leroy et al. have moved the field further, and it will be exciting to see where this leads.

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