Blocking the L-type Ca\textsuperscript{2+} Channel With a Gem
A Paradigm for a More Specific Ca\textsuperscript{2+} Channel Blocker

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Ca\textsuperscript{2+} channel blockers have been an important part of the cardiovascular pharmacological armamentarium for more than 2 decades. These agents were developed as antianginals and antihypertensives, but their indications have expanded to include treatment of certain arrhythmias because of their atrioventricular (AV) nodal blocking properties. Interestingly, the development of these compounds largely preceded our knowledge of the molecular composition and detailed functional properties of voltage-gated Ca\textsuperscript{2+} channels. In fact, Ca\textsuperscript{2+} channel blockers were critical in defining the distinct class of voltage-dependent Ca\textsuperscript{2+} channels referred to as L-type Ca\textsuperscript{2+} channels, which can be found in cardiac myocytes, skeletal myocytes, vascular smooth muscle cells, neurons, and endocrine cells among other cells. Despite this broad distribution of L-type Ca\textsuperscript{2+} channels, Ca\textsuperscript{2+} channel blockers have proven useful agents because they exhibit pharmacological specificity for vascular smooth muscle and cardiac muscle. This specificity is attributable to a variety of factors including the voltage and use-dependent blocking properties of these agents, subtle differences in the sensitivity of distinct isoforms of L-type Ca\textsuperscript{2+} channels present in different tissues, and the tissue distribution of the drugs. In addition, different classes of Ca\textsuperscript{2+} channel blockers vary in their relative potency to block vascular smooth muscle Ca\textsuperscript{2+} channels (antihypertensive/antianginal properties) relative to their block of AV nodal Ca\textsuperscript{2+} channels (antiarrhythmic properties). Alas, the specificity is not perfect, and so these agents can bring with them undesired side effects including constipation, peripheral edema, dizziness, and headache. In addition, problems can arise from the overlap of vascular smooth muscle and cardiac blocking properties. For example, if one wants to control a rapid heart rate in a hypertensive patient with atrial fibrillation by blocking AV nodal Ca\textsuperscript{2+} channels, accompanying vasodilation from vascular smooth muscle blockade can be problematic. Alternatively, treating angina in a patient with left ventricular dysfunction would ideally target coronary artery smooth muscle L-type Ca\textsuperscript{2+} channels without blocking ventricular myocyte Ca\textsuperscript{2+} channels and producing a negative inotropic effect. Even discriminating between different populations of L-type Ca\textsuperscript{2+} channels within the heart is desirable because one might want to prevent an AV nodal reentrant tachycardia but not at the expense of inducing significant sinus bradycardia or negative inotropy. Although new generations of pharmacological Ca\textsuperscript{2+} channel blockers have emerged, ultimate target specificity for desired applications has remained elusive.

In the quest to provide highly localized and specific Ca\textsuperscript{2+} channel blockade, Murata et al, in this issue of Circulation Research, demonstrate a novel approach using gene therapy in proof of principle experiments.\textsuperscript{1} This work exploits the growing understanding of the molecular workings of Ca\textsuperscript{2+} channels and the regulatory processes governing these channels. To understand their study, some background on the molecular properties of the L-type Ca\textsuperscript{2+} channel is necessary. L-type Ca\textsuperscript{2+} channels are composed of 4 subunits including a pore-forming, voltage-gated \(\alpha_1\) subunit (Ca\textsubscript{1,Lx} genes) and auxiliary \(\alpha_2\)-\(\delta\), \(-\beta\), and \(-\gamma\) subunits.\textsuperscript{2} Perhaps the best characterized auxiliary subunit is the cytoplasmic \(\beta\) subunit, which is encoded by 4 separate genes (Ca\textsubscript{\(\beta_1\)}, Ca\textsubscript{\(\beta_2\)}, Ca\textsubscript{\(\beta_3\)}, and Ca\textsubscript{\(\beta_4\)}), each of which can be alternatively spliced.\textsuperscript{3} The Ca\textsubscript{\(\beta\)} subunit has 2 important classes of effects on Ca\textsuperscript{2+} channels, promoting the trafficking of channels to the surface membrane and, secondly, modulating the gating of the channels typically favoring larger currents.\textsuperscript{4,5} Recent functional studies and crystallographic information have defined the Ca\textsubscript{\(\beta\)} subunits as members of the membrane-associated guanylate kinase (MAGUK) family of proteins, with 2 well-conserved protein–protein interaction domains: a Src homology 3 domain and a guanylate kinase domain.\textsuperscript{6–10} The MAGUK proteins are known to be scaffolding proteins, and so it is not surprising that proteins are emerging which interact with Ca\textsubscript{\(\beta\)} subunits including various members of the Rem, Rem1, Rad, and Gem/Kir (RGK) family of Ras-like GTPases.\textsuperscript{11,12} The GTP-bound forms of these RGK proteins actively bind to Ca\textsubscript{\(\beta\)} subunits and result in a strong inhibition of Ca\textsuperscript{2+} currents (Figure).\textsuperscript{11,13} Furthermore, Ca\textsuperscript{2+}/calmodulin binding to Kir/Gem inhibits GTP binding,\textsuperscript{14} suggesting a feedback loop between intracellular Ca\textsuperscript{2+} levels and Ca\textsuperscript{2+} channel function.\textsuperscript{11} Thus, an increasingly complex model is emerging of the L-type Ca\textsuperscript{2+} channel as part of a dynamic macromolecular signaling complex.\textsuperscript{15,16}

Taking advantage of the potent downregulatory effects of Gem on Ca\textsuperscript{2+} channels, Murata et al tested adenoviral delivery of Gem to the heart as a form of a genetic Ca\textsuperscript{2+} calcium channel blocker.\textsuperscript{1} To first examine the ability of their adenoviral construct to reduce L-type Ca\textsuperscript{2+} currents, the authors demonstrated that global adenovirus-mediated delivery of Gem to guinea pig hearts markedly decreased L-type

\textsuperscript{1} The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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Ca\(^{2+}\) channel current (I\(_{\text{Ca,L}}\)) density in isolated ventricular myocytes. Consistent with a reduction in I\(_{\text{Ca,L}}\), there was shortening of action potential duration and the related electrocardiographic corrected QT interval, as well as a reduction in left ventricular systolic function. Next, catheter delivery of the adenoviral Gem via the AV nodal artery was tested in a swine model of atrial fibrillation with a rapid ventricular rate. As predicted, there was a decrease in AV nodal conduction with a clinically significant reduction in ventricular rate. Thus, the authors have provided evidence for very localized Ca\(^{2+}\) channel blockade of clinical significance.

The experiments also provide insights into the basic biology of Gem–Ca\(^{2+}\) channel interactions by investigating the mechanism by which Gem reduces I\(_{\text{Ca,L}}\) (Figure). By measuring intramembrane charge movement (Q) associated with L-type Ca\(^{2+}\) channels in the myocytes, the authors examined for changes in the relative density of channels in the surface membrane. A striking decrease in the maximal amount of charge movement was present in the Gem-treated myocytes, suggesting an important reduction in the density of sarcolemmal L-type Ca\(^{2+}\) channels. This finding is consistent with the studies of Beguin et al., who used immunocytochemistry techniques to also demonstrate a decrease in abundance of membrane Ca\(^{2+}\) channels with Gem expression.\(^{11}\) The effect most intuitively could be attributed to Gem blocking the ability of C\(_{\alpha}\) to bind the Ca\(_{\alpha}\) subunit and, thus, interfering with membrane trafficking. An alternative is that Gem can reduce the stability of the channel in the surface membrane. Murata et al. then argue that there is no clear effect on the gating of the remaining channels based on a similar voltage-dependence of charge movement in treated and untreated cells. However, this is not a certain finding because important changes in voltage-dependent gating can occur even without changes in the Q-V curves, but rather in the coupling of the voltage sensors to channel opening. To assess this, the voltage dependence of I\(_{\text{Ca,L}}\) activation needs to be carefully examined. Based on the I-V curves in their Figure 1, a positive shift in the voltage dependence of current activation by Gem treatment may occur, suggesting that Gem may alter gating. However, it seems likely that in this model, the reduction in abundance of sarcolemmal membrane channels predominates.

Overall, the exciting results of Murata et al.\(^1\) demonstrate a specific genetic Ca\(^{2+}\) channel blocker capable of highly localized targeting, but this pioneering study also raises many important questions before clinical applications can be considered. For example, how can the effect be titrated as too much Ca\(^{2+}\) channel blockade could produce heart block. Additionally, as the authors acknowledge, adenovirus provides only transient expression of the gene product and can evoke undesirable immune consequences; therefore, other delivery vectors will be needed for clinical applications. An additional concern is that even though localized delivery to the AV nodal artery is undertaken, it is still likely that some virus will escape this target. In this regard, it is interesting to note that Gem is not only capable of reducing L-type Ca\(^{2+}\) currents but can also block N and P/Q channels, according to available data. Although the authors provide evidence that I\(_{\text{Ca,L}}\) is specifically reduced compared with other major ionic currents in ventricular myocytes, there remains concern about the effect of Gem on distinct cellular processes. Given the limited understanding of the role of RGK proteins in cardiac cell biology, caution is needed as less desirable effects may occur from overexpression such as alterations in the microtubule network. The bar is set high for gene therapy to provide rate control in atrial fibrillation as many patients tolerate current drugs reasonably well and catheter ablation technology continues to improve. Perhaps treatment of other forms of heart diseases may provide a more ready application for this genetic Ca\(^{2+}\) channel blocker such as hypertrophic cardiomyopathy. Whether this Gem is the diamond in the rough for highly specific, clinically important calcium channel blockade awaits further investigation.

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