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COMMENT

Does cilnidipine, a dual L- and N-type Ca^{2+} blocker, shows promise in drug repositioning approaches?

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Hypertension is the most common chronic disease worldwide and should be treated carefully because the human lifespan is increasing, and many individuals may develop additional complications. As a result of intense investigations by basic and clinical researchers, several different types of drugs can now be used to treat hypertension, such as calcium channel blockers, especially dihydropyridines (DHPs); inhibitors of the renin–angiotensin system; β blockers; and diuretics. The choice of appropriate drugs for each patient is therefore a major concern.

Minato et al. reported in *Hypertension Research* that the L-type and N-type calcium channel blocker cilnidipine may rescue HL-1 cells from hypoxia/reoxygenation injury [1]. Interestingly, the authors used a patch-clamp technique and found that this favorable effect may result from shortening of the action potential duration (APD) due to inhibition of intracellular Ca^{2+} overload and from activation of endothelial nitric oxide synthase (eNOS) and production of nitric oxide (NO). HL-1 cells are cardiac atrial cells originating from mice that, because of their immortalized features, have recently been increasingly employed to investigate the precise mechanisms in cardiomyocytes using either the siRNA technique, overexpression experiments, or the patch-clamp method. However, few studies have adopted an electrophysiological approach to elucidate the effects of calcium channel blockade in HL-1 cells.

The study of Minato et al. has raised a few questions. First, it remains to be elucidated in future studies whether the APD shortening effect directly causes an improved cell survival rate or, alternatively, is a concomitant effect. Second, although the APD shortening effect may result from a reduction in intracellular Ca^{2+}, it is also feasible that it causes micro reentry in the atrium of cardiac muscle, possibly resulting in persistency of atrial fibrillation in patients. Finally, the mechanism by which NO inhibits intracellular Ca^{2+} is not discussed in this article. We will discuss this final question in greater detail.

Although the mechanisms by which NO regulates Ca^{2+} handling are not clear in this article, it has been investigated in detail by other researchers. NO, by its nature and properties, has been recognized as a prototypical endothelium-derived relaxing factor and is generated by three different enzymes, eNOS, neuronal nitric oxide synthase (nNOS), and inducible nitric oxide synthase (iNOS). While nNOS is expressed constitutively in specific neurons of the brain and iNOS in inflammatory tissues, eNOS is essential for the generation of NO to dilate vessels in a healthy cardiovascular system. Furthermore, eNOS and NO are also believed to regulate cardiac function. NO has modulatory effects on the β-adrenergic pathway, leading to the downregulation of L-type Ca^{2+} currents by PKG activation and a decrease in cyclic adenosine monophosphate, cAMP, which is caused by the cyclic guanosine monophosphate (cGMP)-phosphodiesterase-dependent axis [2]. The NO–cGMP–protein kinase G (PKG) pathway also regulates Ca^{2+} release and uptake from the sarcoplasmic reticulum (SR) [3, 4]. The suggested schematic model of NO-related Ca^{2+} handling and signaling is shown in Fig. 1.

The restriction of intracellular Ca^{2+} overload by cilnidipine is of interest since intracellular Ca^{2+} overload is closely associated with atrial fibrillation in patients [5]. Atrial fibrillation can be maintained by rapid focal ectopic activity or reentry. Reentry requires a vulnerable substrate and an initiating trigger. Delayed afterdepolarizations result from abnormal diastolic Ca^{2+} release from the SR via RyR2 Ca^{2+} release channels, whereas early afterdepolarizations result from excessive APD prolongation that allows ICaL to depolarize the cell from the AP plateau. Both of these mechanisms are related to electrophysiologic vulnerability.

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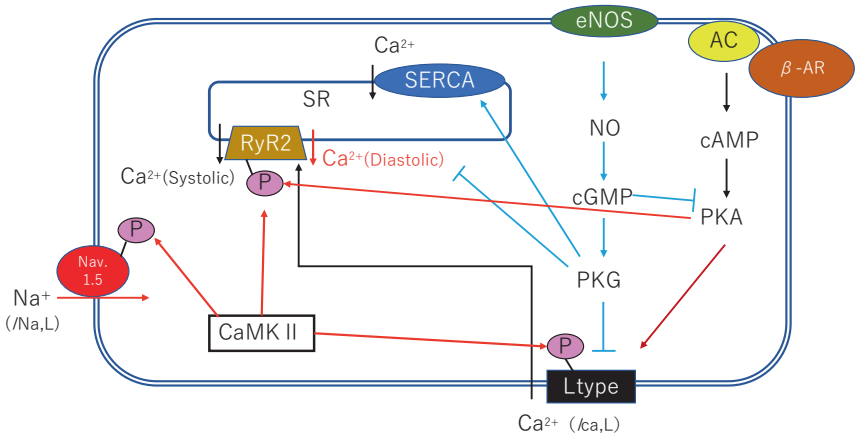

Fig. 1 Schematic model depicting Ca^{2+} handling and suggested modulation by NO. Red line: aberrant Ca^{2+} handling. Blue line: suppression of Ca^{2+} overload by the NO-mediated cascade

Excess Ca^{2+} is removed by NCX (Na^+, Ca^{2+} exchanger) to maintain electric homeostasis; however, excessive Ca^{2+} release may produce an arrhythmogenic depolarizing current [6]. Diastolic SR Ca^{2+} release or RyR2 dysfunction has also been suggested to have a role in intracellular Ca^{2+} overload. Research carried out over the last decade has recently demonstrated the pivotal role of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). CaMKII phosphorylates L-type Ca^{2+} channels, RyR2 receptors, and NaV1.5 Na^+ channels that are responsible for late Na^+ currents, leading to aberrant Ca^{2+} handling [7]. Chronic activation of CaMKII results in cellular remodeling and arrhythmogenic alterations in Ca^{2+} handling and is thought to be upstream of atrial fibrillation receptivity. It has been reported recently that phosphorylation of Ser571 on NaV1.5 by CaMKII results in Ca^{2+} release from the SR or activation of the Na^+/Ca^{2+} exchanger [8, 9]. Increased activity of CaMKII or protein kinase A (PKA) with subsequent uncontrolled diastolic Ca^{2+} release from SR has been suggested to be a central player in calcium handling. Although strong downstream activation of CAMKII is observed, with a vicious circle leading to electrical and structural remodeling of cardiac muscles, intracellular Ca^{2+} overload is another major and important phenomenon for the susceptibility to atrial fibrillation and is also observed in heart failure.

Furthermore, there is evidence that cilnidipine suppresses electrical remodeling and fibrosis of the atrium associated with atrial fibrillation by reducing sympathetic activation. Dogs were kept in atrial fibrillation by right atrial tachypacing for 1 or 3 weeks, leading to the development of atrial fibrillation associated with electrophysiological vulnerability and/or fibrosis in the atria. However, cilnidipine suppressed this electrophysiologic and pathophysiological remodeling associated with atrial fibrillation [10].

Interestingly, cilnidipine restricted the mitochondrial hyperfission-associated myocardial senescence induced by hypoxia [11]. This study showed for the first time that cilnidipine could act as an inhibitor of the interaction between Drp1 and filamin-A. Furthermore, treatment of neonatal rat cardiomyocytes with cilnidipine suppressed mitochondrial hyperfission associated with experimental heart failure caused by exposure to low-dose MeHg, an environmental electrophilic pollutant [12].

Taken together, these results indicate that cilnidipine may have beneficial effects on atrial cardiac muscle with atrial fibrillation independent of its blood-pressure-lowering effect. Although DHPs including nifedipine, amlodipine, azelnidipine, and cilnidipine are lipophilic and are relatively vascular selective because of the higher affinity to the α1 subunit of calcium channel in the vessels than in the heart, Minato et al. confirmed the cilnidipine functioned in HL-1 cells and mouse ventricular cells at therapeutic concentrations. The researchers used mouse cardiac muscles in their article, although it remains to be clarified whether cilnidipine can protect cardiac muscles in humans. However, a future translational study using cilnidipine to block N- and L-type calcium channels and activate eNOS in patients suffering from atrial fibrillation and/or heart failure shows promise.

Compliance with ethical standards

Conflict of interest KN has received grants from Mitsubishi Tanabe; personal fees from MSD, Astellas, Amgen Astellas, AstraZeneca, Eli Lilly, Otsuka, Daiichi Sankyo, Takeda, Boehringer Ingelheim, Bayer, Pfizer, Ono, and Mitsubishi Tanabe; grants from Asahi Kasei, Astellas, Mitsubishi Tanabe, Teijin, Terumo, Boehringer Ingelheim, and Bayer; and scholarships from Bayer, Daiichi Sankyo, Teijin, Astellas, Takeda, and Bristol-Myers Squibb. The other authors declare that they have no conflict of interest.

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