Letter to the Editor

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Ca^{2+}-Calmodulin–Dependent Protein Kinase Phosphorylation of Ryanodine Receptor May Contribute to the β-Adrenergic Regulation of Myocardial Contractility Independently of Increases in Heart Rate

To the Editor:

In a recent article, MacDonnell et al[1] reported that protein kinase A (PKA) phosphorylation of ryanodine receptor (RyR2) (the Ca^{2+} release channel of the sarcoplasmic reticulum [SR]) at Ser2808/09 site does not have a major role in the sympathetic nervous system (SNS) regulation of cardiac function. This conclusion was based on comparing the effect of isoproterenol on ventricular performance, in vivo, in isolated hearts and myocytes, in wild-type mice and in a genetically modified mouse in which Ser2808 of RyR2 was replaced by alanine (S2808A) to prevent PKA-mediated phosphorylation at this site. Isoproterenol produced an increase in cardiac function both in vivo and in isolated hearts, as well as an enhancement in the L-type Ca^{2+} current (I_{CaL}), the amplitude of the Ca^{2+} transient and the excitation–contraction coupling (ECC) gain in isolated myocytes, which were not significantly different between wild-type and S2808A mice.

We have previously demonstrated the lack of functionality of the PKA-dependent phosphorylation of RyR2 at Ser2808/09.[2] In perfused rat hearts, we showed that the isoproterenol-induced phosphorylation of RyR2 was associated with an enhancement of the [^{3}H]-ryanodine binding and the velocity of fast Ca^{2+} release in SR vesicles from the same hearts. This increase in RyR2 activity, however, was not attributable to the PKA-dependent phosphorylation at Ser2809 site. Therefore, the results by MacDonnell et al are in full agreement with our previous findings and confirm in genetically manipulated mice the lack of functionality of the RyR2 Ser 2808/09 site.

The purpose of our letter is to point out, in the first place, an omission of the authors in their discussion that could lead to the erroneous acceptance that Ser2808/09 is the only RyR2 site by which isoproterenol can alter the function of the SR Ca^{2+} release channel. As an example, MacDonnell et al[1] concluded, "Our results strongly support the already well-established idea that PKA mediated increases in I_{CaL}, and increases in SR Ca^{2+} uptake and release are the primary mechanisms by which the SNS increases the rate of force of contraction." (In this context, the meaning of increased SR Ca^{2+} release refers to that produced by the enhanced SR Ca^{2+} uptake/load and not to the increase produced by a change in the RyR2 phosphorylation, which the authors discarded.) The authors further say, "The small ISO-induced increases in ECC gain that we observed at negative test potentials are well explained by the fact that ISO increases the open probability of I_{CaL}." In both cases, they are excluding, without experimental support, any possible participation of isoproterenol-induced RyR2 phosphorylation different from Ser2808/9 site in the positive inotropic effect of isoproterenol.

Experiments by Xiao et al[3] suggested that Ser2030 site of the RyR2, and not Ser2809, might be the only possible PKA-dependent functional site at the RyR2 level. Moreover, we demonstrated in our previous work[2] that calmodulin kinase (CaMK)II-dependent phosphorylation of the RyR2 at Ser2815 site was responsible for the β-adrenergic–induced increase in the channel activity. Whether this increase in RyR2 activity plays a significant role in the positive inotropic action of isoproterenol seems to be still open to debate and should continue to be a matter of discussion. We emphasize that although the above quoted statements may be correct, they are incomplete and may cause confusion in an already difficult subject.

The second purpose of our letter is to indicate that we disagree with a concept that MacDonnell et al[1] put forward in their discussion that suggests that the only mechanism by which isoproterenol may produce an increase in CaMKII activation is by increasing heart rate, ie, referring to their in vivo experiments the authors express: "The potential shortcoming of these experiments is that the ISO-induced increase in heart rate may activate Ca^{2+}-regulated signaling pathways (particularly those involved Ca^{2+}/calmodulin-kinase II). . . " Evidence has grown supporting a critical role of CaMKII in the effects induced by both acute and chronic β-adrenoceptor stimulation, including the results mentioned above.[2,4–7] These effects, however, occur without the requisite of a β-adrenergic–induced increase in heart rate. Thus, the prevalent concept that direct PKA phosphorylation is solely responsible for β-adrenoceptor–mediated cardiac cellular responses has been challenged by abundant experimental evidence.

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Disclosures

None.

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