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Effect of hypertrophic mutations of cardiac myosin-binding protein C on actin-myosin interaction

Kochurova A.[1], Beldiia E.[1, 2], Matyushenko A.[3], Bershitsky S.[1], Kopylova G.[1]*, Shchepkin D.[1]

[1] *Institute of Immunology and Physiology, UB RAS, Yekaterinburg, Russia*

[2] *UrFU, Ekaterinburg, Russia*

[3] *Federal Research Center of Biotechnology, RAS, Moscow, Russia*

** g_rodionova@mail.ru*

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Motivation and Aim: About 45 % of sarcomere protein mutations associated with hypertrophic cardiomyopathies (HCM) are cardiac myosin binding protein C (cMyBP-C). The molecular mechanisms of the development of HCM with point mutations in cMyBP-C are practically not studied. We studied the effect of HCM mutations D75N, P161S, and L352P in the N-terminal part of the cMyBP-C on the actin-myosin interaction in the myocardium.

Methods: Myosin was obtained from the left ventricle of the pig heart. C0-C2 fragment of wild-type (WT) human cMyBP-C and with HCM mutations D75N, P161S, and L352P, human cardiac tropomyosin and troponin were expressed in *E. coli*.

The effect of mutations on actin-myosin interaction was studied in an *in vitro* motility assay. The calcium dependence of the sliding velocity of thin filaments, reconstructed from F-actin, tropomyosin, and troponin, was fitted by the Hill equation: $V = V_{max} \times (1+10^{n(pCa-pCa50)})^{-1}$, where V and V_{max} are a velocity and the maximum velocity at saturating calcium concentration respectively; $p\mathrm{Ca}_{50}$ (i.e., calcium sensitivity) is pCa at which half-maximal velocity is achieved; and n is the Hill cooperativity coefficient.

Results: We found that 500 nM P161S C0-C2 fragment increased the maximum sliding velocity of thin filaments at a saturating Ca^{2+} concentration by 30 %, and mutations D75N and L352P reduced it by 20 and 70 % compared to the WT C0-C2 fragment. The P161S and D75N mutations significantly reduced the Ca^{2+} sensitivity of the actin-myosin interaction. At 500 nM L352P C0-C2, the filament velocity did not depend on the Ca^{2+} concentration. At 300 nM L352P C0-C2, this dependence was sigmoidal, its $p\mathrm{Ca}_{50}$ was lower than with 300 nM WT C0-C2; and mutation L352P did not inhibit the filament sliding at low Ca^{2+} concentrations. The P161S and D75N mutations impaired thin filament activation by increasing the myosin concentration at which filament velocity is half-maximal.

Then we studied the effect of a specific activator of ventricular myosin, omecamtiv mecarbil (OM) [1, 2], on actin-myosin interaction in the presence of the P161S cMyBP-C mutation. Previously, it was shown that OM increases the force of multicellular preparations from the left ventricle at non-saturating calcium concentration [3, 4]. We hypothesized that OM could neutralize the decrease in the Ca^{2+} sensitivity of actin-myosin interaction. We found that in the absence of C0-C2 fragment, OM halved the maximum sliding velocity of thin filaments and increased the Ca^{2+} sensitivity by 0.2 pCa. With WT C0-C2 fragment, OM reduced maximum filament velocity to a lesser

extent, only by 30 %, and significantly increased Ca^{2+} sensitivity. With P161S C0-C2 fragment, OM decreased the filament velocity by 30 %, but calcium sensitivity did not change.

Conclusion: The studied HCM cMyBP-C mutations disrupt the calcium regulation of actin-myosin interaction, which may be one of the molecular mechanisms of HCM development. The P161S mutation impairs the activation of thin filaments, and OM only partially neutralizes the negative effect of this mutation on the actin-myosin interaction.

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References

1. Teerlink J.R. A novel approach to improve cardiac performance: cardiac myosin activators. *Heart Fail Rev*. 2009;14(4):289-298. doi 10.1007/s10741-009-9135-0
2. Felker G.M., Solomon S.D., Claggett B. et al. Assessment of omecamtiv mecarbil for the treatment of patients with severe heart failure: a post hoc analysis of data from the galactic-hf randomized clinical trial. *JAMA Cardiol*. 2022;7(1):26-34. doi 10.1001/jamacardio.2021.4027
3. Nagy L., Kovács Á., Bódi B. et al. The novel cardiac myosin activator omecamtiv mecarbil increases the calcium sensitivity of force production in isolated cardiomyocytes and skeletal muscle fibres of the rat. *Br J Pharmacol*. 2015;172(18):4506-4518. doi 10.1111/bph.13235
4. Mamidi R., Li J., Gresham K.S. et al. Dose-dependent effects of the myosin activator omecamtiv mecarbil on cross-bridge behavior and force generation in failing human myocardium. *Circ Heart Fail*. 2017;10(10):e004257. doi 10.1161/CIRCHEARTFAILURE.117.004257