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Influence of tropomodulin 1 on calcium regulation of actin-myosin interaction in the ventricles

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Introduction and Aim: For the contractile function of the myocardium, a strictly ordered and stable structure of the contractile apparatus of the cardiomyocyte is important determined by a number of proteins, which include tropomodulin (Tmod) [1]. Tmod binds to the thin filament and prevents it from disassembling and attaching new actin globules [1, 2]. Recently, it was found that in skeletal muscles, Tmod is involved in the activation of the thin filament, the regulation of actin-myosin interaction and affects the force developed by muscles [3]. The aim of our work was to study the molecular mechanism of the effect of the cardiac isoform of tropomodulin, Tmod1, on the calcium regulation of the actin-myosin interaction underlying myocardial contraction.

Methods: Myosin and native thin filaments (NTF) consisting of actin, troponin, and tropomyosin were isolated from the left ventricle of sheep. Human Tmod1 was expressed in *E. coli* [4]. To study the effect of Tmod1 on actin-myosin interaction we used an *in vitro* motility assay [4]. The calcium dependence of the sliding velocity of NTF over myosin in the *in vitro* motility assay was approximated by the Hill equation: $V = V_{max} \times (1+10^{h(p\mathrm{Ca}-p\mathrm{Ca}50)})^{-1}$, where V and V_{max} are the NTF velocity and the maximum velocity at a saturating calcium concentration; $p\mathrm{Ca}_{50}$ – calcium sensitivity, pCa value at which half of V_{max} is reached; h is the Hill coefficient. To study the effect of Tmod1 on the calcium regulation of actin-myosin interaction, a concentration of 500 nM of Tmod1 was used in accordance with previous studies [5]. Data are presented as mean ± standard deviation. Differences were assessed using the Mann–Whitney U-test, $p < 0.05$.

Results: It was found that Tmod1 affects the cooperative mechanisms of calcium regulation of actin-myosin interaction in the myocardium. Tmod1 significantly reduced the calcium sensitivity of sliding velocity of NTF from $p\mathrm{Ca}_{50}$ 5.99±0.01 to $p\mathrm{Ca}_{50}$ 5.65±0.01. Tmod1 statistically insignificantly increased the Hill cooperativity coefficient from 1.66±0.11 to 1.78±0.21. Tmod1 did not affect the NTF sliding velocity at saturating calcium concentration. The maximum sliding velocity of NTF without Tmod1 was 2.3 ± 0.1 μm/s, while with the addition of Tmod1 it was 2.2 ± 0.1 μm/s.

Conclusion: Thus, Tmod1 can act as a new regulator of actin-myosin interaction in the myocardium. The results obtained will contribute to understanding the molecular mechanisms of the influence of Tmod on the activation of a thin filament and actin-myosin interaction in the myocardium and the search for new therapeutic targets in heart pathologies.

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