Epigenetic regulation of exercise-improved LTCC and BKCa channels function in hypertension mesenteric arteries

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Objective To investigate the epigenetic mechanism of the voltage-gated L-type Ca$^{2+}$ channel (LTCC) and the large-conductance Ca$^{2+}$-activated K$^+$ channel (BK$_{Ca}$) function in mesenteric arterial myocytes improved by regular aerobic exercise in hypertension.

Methods 12-week-old male SHR and WKY rats were randomly assigned to sedentary and exercise training groups, respectively. Exercise groups were performed a moderate-intensity treadmill running. After 8 weeks, patch clamp study, Ca$^{2+}$ image, Western blot, qPCR, bisulfite sequencing PCR were used to detect the LTCC and BK$_{Ca}$ channel currents, BK$_{Ca}$ single channel gating properties, Ca$^{2+}$ spark, mRNA and protein expression of LTCC $\alpha_{1c}$ together with BK$_{Ca}$ $\alpha$ and $\beta$1 subunits, DNA methylation level of $\alpha_{1c}$ and $\beta$1 gene promoter region, miR-328 expression. In vitro experiment, miR-328 mimic and miR-328 inhibitor were transfected into cultured arterial myocytes to make miR-328 overexpressing or silencing, the mRNA and protein expression of $\alpha_{1c}$ subunits were determined after 48 h transfection.

Results 1) After 8 weeks of exercise, SBP in both exercise groups of WKY and SHR were significantly lower than that of their sedentary counterparts. 2) Exercise normalized the increased LTCC and BK$_{Ca}$ current density of mesenteric arterial myocytes in SHR. 3) Exercise attenuated the increased single BK$_{Ca}$ channel open Probability ($P_o$) and the amplitude of Ca$^{2+}$ spark in hypertension. 4) Exercise inhibited the upregulated mRNA and protein expression of BK$_{Ca}$ $\beta$1 subunit in mesenteric arteries from SHR; $\beta$1 gene promoter was demethylation in hypertension, exercise increased the methylation level at $\beta$1 gene promoter of SHR. 5) The protein expression of LTCC $\alpha_{1c}$ subunit was significantly increased in SHR, while decreased by exercise; the expression of miR-328 in mesenteric arteries was highly negative correlation with $\alpha_{1c}$ subunit. 6) The miR-328 overexpression by transfecting miR-328 mimic decreased $\alpha_{1c}$ subunit protein level significantly, while miR-328 inhibitor made $\alpha_{1c}$ subunit a slight increase.

Conclusions Regular aerobic exercise efficiently reduces blood pressure of SHR, enhances $\beta$1 gene promoter methylation, mediates miR-328 inhibiting the $\alpha_{1c}$ expression at post-transcriptional level, which might be the epigenetic mechanism underlying exercise-improved LTCC and BK$_{Ca}$ channels function in mesenteric arteries of hypertension.