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C1q/tumor necrosis factor-related protein-3 enhances the contractility of cardiomyocyte by increasing calcium sensitivity

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ABSTRACT

C1q/tumor necrosis factor-related protein-3 (CTRP3) is an adipokine that protects against myocardial infarction-induced cardiac dysfunction through its pro-angiogenic, anti-apoptotic, and anti-fibrotic effects. However, whether CTRP3 can directly affect the systolic and diastolic function of cardiomyocytes remains unknown. Adult rat cardiomyocytes were isolated and loaded with Fura-2AM. The contraction and Ca^{2+} transient data was collected and analyzed by IonOptix system. 1 and 2 μg/ml CTRP3 significantly increased the contraction of cardiomyocytes. However, CTRP3 did not alter the diastolic Ca^{2+} content, systolic Ca^{2+} content, Ca^{2+} transient amplitude, and L-type Ca^{2+} channel current. To reveal whether CTRP3 affects the Ca^{2+} sensitivity of cardiomyocytes, the typical phase-plane diagrams of sarcomere length *vs.* Fura-2 ratio was performed. We observed a left-ward shifting of the late relaxation trajectory after CTRP3 perfusion, as quantified by decreased Ca^{2+} content at 50% sarcomere relaxation, and increased mean gradient (μm/Fura-2 ratio) during 500–600 ms (-0.163 *vs.* −0.279), 500–700 ms (-0.159 *vs.* −0.248), and 500–800 ms (-0.148 *vs.* −0.243). Consistently, the phosphorylation level of cardiac troponin I at Ser23/24 was reduced by CTRP3, which could be eliminated by preincubation of okadaic acid, a type 2A protein phosphatase inhibitor. In summary, CTRP3 increases the contraction of cardiomyocytes by increasing the myofilament Ca^{2+} sensitivity. CTRP3 might be a potential endogenous Ca^{2+} sensitizer that modulates the contractility of cardiomyocytes.

1. Introduction

Myocardial contractility is primarily regulated by Ca^{2+} cycling into and out of the cytoplasm of cardiomyocytes [1,2]. Apart from the amplitude and duration of Ca^{2+} transient, the alteration in myofilament Ca^{2+} sensitivity can also impact cardiac contractility [3]. Adipokines, which are mainly produced by adipose tissue, play various roles in regulating metabolic and cardiovascular systems [4]. Several adipokines have been proved to regulate the contractility and Ca^{2+} dynamics in cardiomyocytes. For example, adiponectin and apelin promotes the contraction of diabetic or failing cardiomyocyte through increasing Ca^{2+} availability [5,6], whereas leptin, resistin, interleukin-1β, tumor necrosis factor-α, and fatty acid-binding protein suppress the contractile performance of cardiomyocyte through inhibiting the Ca^{2+} dynamics [7–10].

C1q/tumor necrosis factor-related protein-3 (CTRP3) is an adipokine that expressed in differentiated adipocyte, cartilage, placenta, colon, small intestine, heart, lung, kidney, and spleen [11]. The biological function of CTRP3 has been related to cartilage development, osteoclastogenesis, glucose and lipid metabolism, inflammatory response, angiogenesis, vascular calcification, and mitochondrial biogenesis, etc [12–20]. Recently, CTRP3 has aroused more concerns because of its cardio-protective effects. Circulating CTRP3 is decreased in patients of obesity, diabetes, myocardial infarction (MI), acute coronary syndrome, and stable angina pectoris [16,18,21–25]. Replenishment of CTRP3 by intraperitoneal pumping of recombinant protein or local injection of CTRP3 adenovirus can improve cardiac function and inhibit ventricular remodeling in rodent model of MI. The underlying mechanism for the cardio-protective effects of CTRP3 involves its pro-angiogenic,

Abbreviations: CTRP3, C1q/tumor necrosis factor-related protein-3; cTnI, cardiac troponin I; cTnC, cardiac troponin C; I_{Ca}, L-type Ca^{2+} channel current; MI, myocardial infarction; PP2A, type 2A protein phosphatase.

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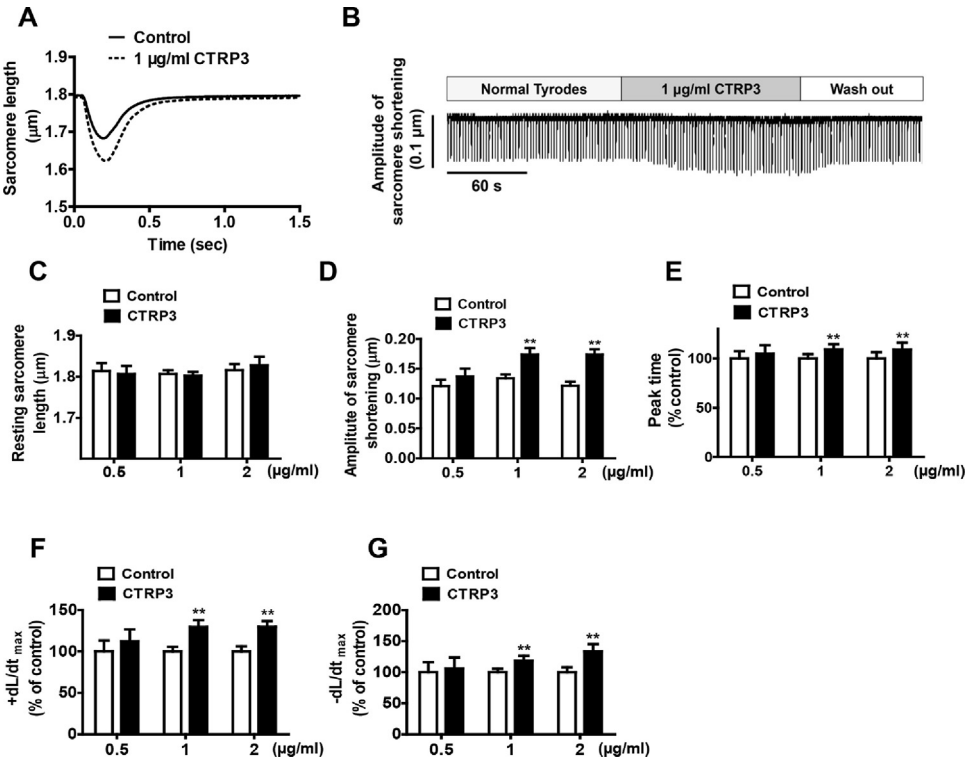

Fig. 1. Effects of CTRP3 on cardiomyocyte shortening.
Cardiomyocytes were perfused with perfusion solution (Tyrode's solution containing 1 mM $CaCl_2$) for 2 min, followed by CTRP3 perfusion for 2 min, then CTRP3 was washout. (A and B) were the representative trace (A) and original trace (B) of sarcomere shortening by 1 μg/ml CTRP3 perfusion. (C-G) represented the quantitative analysis of diastolic sarcomere length (C), contraction amplitude (D), peak time (E), maximum systolic velocity (+dL/dt$_{max}$) (F), and maximum diastolic velocity (-dL/dt$_{max}$) (G). $^{**}P < 0.01$ *vs.* control. n = 13–18 cardiomyocytes from 6 hearts.

anti-apoptotic, and anti-fibrotic effects in myocardium [16,18]. Besides, our previous study demonstrated that CTRP3 promotes ATP generation in cardiomyocytes through modulating mitochondrial biogenesis, and protects against hypoxia/reoxygenation injury by ameliorating mitochondrial dysfunction [19]. Although the above studies may partly explain the protective effect of CTRP3 in cardiac dysfunction, whether CTRP3 can directly improve the contraction of cardiomyocytes is still unknown. The present study was designed to investigate the role of CTRP3 in the contractility, Ca^{2+} dynamics, and myofilament Ca^{2+} sensitivity of cardiomyocytes.

2. Materials and methods

2.1. Reagents

Human recombinant globular CTRP3 (A00082-01) was purchased from Aviscera Bioscience (Santa Clara, CA, USA). Type II collagenase (LS004176) was purchased from Worthington Biochemical (Lakewood, NJ, USA). Fura-2/AM (F-1201) was purchased from Invitrogen (Carlsbad, CA, USA). Propranolol hydrochloride (H26645) was purchased from Alfa Aesar (Ward Hill, MA, USA). Isoproterenol hydrochloride (#16379) and okadaic acid (O9381) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Antibody for cardiac troponin I (cTnI, MMS-416R, RRID: AB_10719261) was from Covance (Emeryville, CA, USA). Antibody for phospho-cTnI (Ser23/24) (#4004, RRID: AB_2206275) was purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies for phospho-cTnI (Ser43) (ab59420, RRID: AB_942207) and type 2A protein phosphatase (PP2A, ab33537, RRID: AB_777384) were purchased from Abcam (Cambridge, MA, USA). Antibody for phospho-PP2A (Tyr307, BS4867) was purchased from Bioworld Technology (Minneapolis, MN, USA).

2.2. Isolation of adult rat ventricular myocytes

The investigation was conformed to the EU Directive 2010/63/EU for animal experiments. All the experimental procedures were approved by the Ethics Committee of Animal Research, Peking University Health Science Centre. Briefly, male Sprague-Dawley rats weighing between 180 and 220 g were anesthetized with 4% isoflurane-95% O_2 and sacrificed. Hearts were rapidly removed and placed in ice cold Tyrode's solution (in mM: 137 NaCl, 5.4 KCl, 10 glucose, 20 HEPES, 1.2 $MgCl_2$, and 1.2 NaH_2PO_4, pH 7.4). Following the aortic cannulation, hearts were perfused at 37 °C using a Langendorff's apparatus with isolation solution (Tyrode's solution with 20 mM taurine) for 8 min. Then, hearts were perfused with digestive solution (Tyrode's solution with 20 mM taurine, 1 mg/ml BSA, 50 μM $CaCl_2$, and 250 U/ml type II collagenase) at 37 °C for 20 min. Left ventricle was removed in a new 5 ml digestive solution and cut into small pieces. The above solution was then agitated in a water bath at 37 °C for 5 min the supernatant was collected carefully and filtered through 400 μm nylon mesh. Dissociated cells was centrifuged at 500*g* for 45 s, cells was resuspended with suspension solution (Tyrode's solution with 20 mM taurine, 1 mg/ml BSA, and 50 μM $CaCl_2$). Cells were gradually resuspended with 0.25, 0.5, 0.75, and 1 mM Ca^{2+} Tyrode's solution for 10 min to recover the extracellular Ca^{2+}.

2.3. Simultaneous measurement of Ca^{2+} transient and sarcomere shortening

Cells were incubated with Fura-2AM (2 μg/ml) in perfusion solution (Tyrode's solution with 1 mM $CaCl_2$) at 25 °C for 30 min, the loaded myocytes were resuspended with a new perfusion solution and rested for at least 30 min to wash out residual Fura-2