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Modulation of Nitric Oxide on Calcium Homeostasis in Isolated Skeletal Myocytes from Zebrafish Larvae

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The excitation-contraction coupling (ECC) is the process that explains the muscle contraction mediated by membrane depolarization, calcium release, force production and calcium reuptake. During each muscle contraction, nitric oxide (NO) is produced via neuronal isoform of nitric oxide synthase. However, the role of NO in the entire process of ECC is still unknown. Meanwhile, in recent decades, zebrafish was more and more extensively used in researches as a vertebrate model organism because of its unique advantages. In this study, we investigated the effects of NO in intact skeletal muscle and isolated skeletal myocytes of zebrafish larvae, to further understand the mechanism of NO in skeletal muscle.

The 5-7 dpf zebrafish larvae were applied for stimulation-mechanical experiments. Under electrical field stimulations, the force-frequency relationship and the effects of NO donor and NOS inhibitor on contractile function of intact skeletal muscle were explored. To understand the effects of NO on Ca^{2+} transients during EC-coupling, we established a simple protocol to isolate the single skeletal myocyte from zebrafish larvae. The pharmacological agents including NO donor (SNAP), NOS inhibitor (L-NAME), DHPR inhibitor (Verapamil), RyR inhibitor (Ryanodine), RyR activator (Caffeine), SERCA inhibitor (CPA), NCX inhibitor ($NiCl_2$), sGC inhibitor (ODQ) and sGC activator (Bay-41) were used to determine the effects and mechanism of NO in isolated skeletal myocytes through Ca^{2+} imaging experiments. Furthermore, the distributions of nNOS, DHPR, RyR, and SERCA in skeletal myocytes of zebrafish larvae and their spatial relationships were observed through immunocytochemistry experiments under confocal laser scanning microscope.

Our findings showed that NO exerted negative modulations on both contractile function of skeletal muscle and Ca^{2+} homeostasis of isolated skeletal myocytes from zebrafish larvae. Moreover, these negative modulations were attributed to NO's inhibitions on RyR and NCX of skeletal myocytes through S-nitrosylation pathway. Through immunocytochemistry experiments, nNOS was shown to distribute widely in the skeletal myocytes but most amounts were assembled in T-tube system and some intracellular organelles which were suspected to be mitochondria. DHPR was also highly concentrated in T-tube system. Furthermore, some of RyR and SERCA distributed along the T-tube, but they were mainly assembled in the edge area of the skeletal myocytes, just under the plasma membrane.

In summary, the present study demonstrates the negative effects and mechanism of NO on contractile function and Ca^{2+} homeostasis in skeletal muscle of zebrafish larvae. The zebrafish as animal model has been used for the researches of

neuromuscular diseases, and here we open a new field of research where functional analysis, such as protocols for force measurement and Ca^{2+} imaging, has been studied.