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TPC2-mediated Ca^{2+} signaling is required for the establishment of synchronized activity in developing zebrafish primary motor neurons.

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ABSTRACT

During the development of the early spinal circuitry in zebrafish, spontaneous Ca^{2+} transients in the primary motor neurons (PMNs) are reported to transform from being slow and uncorrelated, to being rapid, synchronized and patterned. In this study, we demonstrated that in intact zebrafish, Ca^{2+} release +via two-pore channel type 2 (TPC2) from acidic stores/endolysosomes is required for the establishment of synchronized activity in the PMNs. Using the SAIGFF213A;UAS:GCaMP7a double-transgenic zebrafish line, Ca^{2+} transients were visualized in the caudal PMNs (CaPs). TPC2 inhibition via molecular, genetic or pharmacological means attenuated the CaP Ca^{2+} transients, and decreased the normal ipsilateral correlation and contralateral anti-correlation, indicating a disruption in normal spinal circuitry maturation. Furthermore, treatment with MS222 resulted in a complete (but reversible) inhibition of the CaP Ca^{2+} transients, as well as a significant decrease in the concentration of the Ca^{2+} mobilizing messenger, nicotinic acid adenine diphosphate (NAADP) in whole embryo extract. Together, our new data suggest a novel function for NAADP/TPC2-mediated Ca^{2+} signaling in the development, coordination, and maturation of the spinal network in zebrafish embryos.

Keywords

NAADP, TPC2, Ca^{2+} signaling, Spinal circuitry, Zebrafish, Acidic store.

INTRODUCTION

During early zebrafish (*Danio rerio*; Hamilton, 1822) development (i.e., starting at ~17.5 hpf), the primary motor neurons (PMNs) begin to display spontaneous and stochastic Ca^{2+} activity (Muto et al., 2011; Warp et al., 2012), which coincides with the generation of Ca^{2+} transients in the slow muscle cells (SMCs; Brennan et al., 2005) and spontaneous SMC-mediated coilings of the trunk (Saint-Amant and Drapeau, 1998). As development proceeds, this early motor behavior matures into an organized form of swimming (Naganawa and Hirata, 2011). This transition requires the establishment of a synchronized, correlated connectivity within the spinal network and with the developing cells of the myotome. A better understanding of the signaling elements that pattern the nascent nervous system will help in deciphering the complexity of adult behaviour (Wilson et al., 2002). The development of the genetically-encoded Ca^{2+} indicator, GCaMP, has greatly advanced our