Spontaneous Ca^{2+} fluctuations mediated by TRPM7 channels in growth plate chondrocytes

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During embryonic bone development, condensations of mesenchymal progenitor cells give rise to round chondrocytes. Subsequently, round chondrocytes proliferate, differentiate into columnar chondrocytes, and then further differentiate into hypertrophic chondrocytes. Ca^{2+} signaling may play essential roles in this process. However, Ca^{2+} handling is largely unknown in growth plate chondrocytes. In this study, we developed a new fluorometric imaging method to survey Ca^{2+} signaling in growth plate chondrocytes using femoral bone slice preparations from E17.5 mouse embryos. Weak Ca^{2+} rises and falls, named spontaneous Ca^{2+} fluctuations, were observed in appreciable populations of round and columnar chondrocytes. Ca^{2+} removal and non-specific Ca^{2+} channel inhibitor Gd^{3+} addition abolished the Ca^{2+} fluctuations. On the other hand, sarco/endoplasmic reticulum Ca^{2+} -pump inhibitors, thapsigargin and cyclopiazonic acid activated the Ca^{2+} fluctuations. Therefore, we confirmed that Ca^{2+} entry is essential for generating the Ca^{2+} fluctuations. Microarray analysis was conducted to identify the Ca^{2+} channel responsible for the Ca^{2+} fluctuations. We found that transient receptor potential melastatin subfamily 7 (TRPM7) channel, a non-selective cation channel that can conduct Ca^{2+} and Mg^{2+} , is expressed in growth plate chondrocytes. Reported TRPM7 inhibitors, FTY720 and NS8593, attenuated the Ca^{2+} fluctuations while TRPM7 activators, naltribe and NNC550396, facilitated the Ca^{2+} fluctuations. Furthermore, the Ca^{2+} fluctuations were depressed by phospholipase C (PLC) inhibitor U73122, while they were stimulated by the large-conductance calcium-activated- K^{+} (BK) channel activator NS1619. Therefore, we conclude that TRPM7 channels mediated spontaneous Ca^{2+} fluctuations and were functionally coupled by PLC and BK channels in growth plate chondrocytes. Currently, we are trying to clarify the physiological function of the spontaneous Ca^{2+} fluctuations mediated TRPM7 channels in bone development.