INTRACELLULAR Ca^{2+} DYNAMICS MODULATION BY ISTAROXIME AND ITS METABOLITE IN PULMONARY ARTERY SMOOTH MUSCLE CELLS

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Abstract:
Smooth muscle cells (SMCs) show phenotypic changes in response to vascular diseases, associated with alterations in Ca^{2+} handling proteins, including SERCA and proteins involved in the store-operated Ca^{2+} entry (SOCE). At the cardiac level, istaroxime acts as a luso-inotropic agent able to inhibit the Na^+/K^+ ATPase and stimulate SERCA2a activity. Its metabolite, PST3093, selectively stimulates SERCA2a.

This project aims to investigate the effects of istaroxime and PST3093 on intracellular Ca^{2+} dynamics in rat pulmonary artery smooth muscle cells (rPASMCs).

First of all, the expression levels of α-SMA, SERCA, and phospholamban (PLN) in rPASMCs were assessed. The effects on intracellular Ca^{2+} dynamics were evaluated by using Fluo4-AM or Fura2-AM Ca^{2+} probes. Specifically, SOCE and ATP-induced sarcoplasmic reticulum (SR) Ca^{2+} release were evaluated in isolated cells through Fluo4-AM, while resting Ca^{2+} levels were analyzed through a cell population study through Fura2-AM. Finally, the inhibition of Na^+/K^+ ATPase current (I_{NaK}) was assessed in voltage-clamped rPASMCs.

In cultured rPASMCs, SERCA2b was the main expressed SERCA isoform; PLN was not detectable. Istaroxime reduced resting Ca^{2+} and SOCE, while it did not affect the amplitude and kinetic of ATP-induced Ca^{2+} transient; finally, I_{NaK} inhibition potency was lower in comparison to cardiac preparations. All these effects were not shared by PST3093.These results suggest that istaroxime affects rPASMCs Ca^{2+} dynamics through mechanisms not related to SERCA2a stimulation and potentially related to SOCE inhibition.