

## NK-receptors, Substance P, Ano1 expression and the ultrastructural features of the muscle coat are modified in the Cav-1<sup>-/-</sup> mouse ileum

Gianluca Cipriani, Maria Simonetta Fausson-Pellegrini, Maria Giuliana Vannucchi

Dipartimento di Anatomia, Istologia e Medicina Legale, Università di Firenze, Italia

Caveolin (Cav)-1 is an integral membrane protein of caveolae playing a crucial role in various signal transduction pathways. Caveolae represent the sites for calcium entry and storage especially in smooth muscle cells (SMC) and interstitial cells of Cajal (ICC). Cav-1<sup>-/-</sup> mice lack caveolae and show abnormalities in pacing and contractile activity of the small intestine. In particular, the absence of caveolae in ICC compromised their ability to maintain frequencies of contraction.

Presently, we investigated, by transmission electron microscopy (TEM) and immunohistochemistry, whether the absence of Cav-1 in Cav-1<sup>-/-</sup> mouse small intestine affects ICC, SMC and neuronal morphology, the expression of NK1 and NK2 receptors, and of Ano1 (also called Dog1 or TMEM16A) an essential molecule for slow wave activity in gastrointestinal muscles. ICC were also labeled with c-Kit and tachykinergic neurons with Substance P (SP).

Immunohistochemical results showed that in Cav-1<sup>-/-</sup> mice: i) ICC were Ano1-negative but maintained c-Kit expression, ii) NK1 and NK2 receptor immunoreactivity was increased and, in the SMC, mainly intracytoplasmatic, iii) SP-immunoreactivity was significantly reduced. Under TEM: i) ICC and SMC lacked typical caveolae but had few and large flask-shaped vesicles we called large-sized caveolae; ii) SMC and ICC contained an extraordinary high number of mitochondria; iii) neurons were unchanged.

In conclusion, the present study shows important changes in SMC, ICC and neurons of the Cav-1<sup>-/-</sup> mice. Loss of Ano1 expression in the ICC and rearrangement of NK receptors in the SMC are interpretable as consequence of Cav-1/caveolae loss and possibly responsible for the impaired contractile activity. However, the impressive richness in mitochondria and the decrease in SP content might represent the ways to compensate the reduced calcium availability and the increased expression of NK<sub>r</sub>, allowing the maintenance of a certain cell function.

Keywords: caveolin-1, immunohistochemistry, electron microscopy, interstitial cells of Cajal, smooth muscle cells.