5. CONCLUSION

Tight junction is a dynamic and complex intercellular structure that switches between “open” and “closed” states according to the needs of the organisms. In recent years significant progress has been achieved in the identification of molecular components of TJ complex, but signalling pathways regulating TJ assembly and function remain poorly understood. A decade ago phorbol esters, potent PKC activators, were recognised as strong modulators of TJ function, although the TJ targets of diacylglycerol/PKC signalling were not identified. A large number of the reports about the effects of phorbol esters on barrier function of TJs appeared to be contradictory: phorbol esters were reported both to improve and to deteriorate barrier properties of epithelial and endothelial cell monolayers. This paradox was not resolved by the application of pan-PKC inhibitors: similar to the effects of phorbol esters, pan-PKC inhibitors were reported both to prevent TJ disassembly and to block TJ formation.

The present study provides a rationale for the contradictory observations mentioned above. Using various isotype-specific PKC inhibitors we were able to show that TJ assembly is regulated by antagonistic signalling of novel and classical/μ PKC isozymes: the formation of TJ complex is favourable when novel PKCs (presumably PKC-δ) are activated and classical PKC/PKCμ is blocked. The inability of the cell to maintain this balance leads to the disassembly of TJs.

Further, for the first time occludin, an important functional and structural transmembrane constituent of TJs, was shown to be a target for PKC-mediated signalling: activation of novel PKCs induced the rapid phosphorylation of occludin with a concomitant translocation to the regions of cell-cell contact. Both the phosphorylation of occludin and its incorporation into the nascent tight junctions was counteracted by classical PKC/PKCμ signalling. *In vitro* experiments showed that the recombinant COOH-terminal domain of murine occludin could be phosphorylated by purified protein kinase Cδ, suggesting that this isoform may regulate tight junction assembly by direct phosphorylation of occludin .

In addition, a simple procedure for the isolation of a low abundance fraction of highly phosphorylated occludin from rat liver was developed, providing the opportunity to study post-translational modifications in the specific fraction of occludin that is incorporated into the tight junctional complex and linked to the cytoskeleton. Using peptide mass fingerprint analysis and electrospray ionization tandem mass-spectroscopy, Ser371 of occludin was for the first time

identified as an *in vivo* phosphorylation site, providing a possibility to study a physiological role of occludin phosphorylation in tight junction physiology.

The present work represents a significant step towards the understanding of the mechanisms regulating tight junction assembly and disassembly and opens many new possibilities for the future studies of tight junction physiology.