5. Summary

It is becoming increasingly clear that cell volume regulatory mechanisms are critically involved in the regulation of a wide variety of physiological functions including cell proliferation and programmed cell death (apoptosis). In the current study, human hepatoma cell line (HepG2) was chosen as a system to investigate hypertonicity-induced cation channels (HICCs) and their role in hepatocytes proliferation.

Hypertonicity-induced cation conductance is the main mechanism of RVI, which is a restoration process of cell volume after osmotic cell shrinkage. Electrophysiological techniques were used to monitor the activation of HICC current and its pharmacological characteristics in HepG2 cells. In the present study, it was demonstrated that HICC current is inhibited by amiloride, flufenamate and Gd$^{3+}$ in a dose-dependent manner. Next, the concentration dependency of the effects of the same pharmacological compounds on HepG2 cell proliferation was examined. The observed rate of proliferation exhibited a pharmacological profile that was very similar to that of channel inhibition. This was already indicative that the cation channels of RVI may actually function as mediators of proliferation.

With the aid of siRNA technique and whole-cell patch-clamp recordings α-subunit of epithelial sodium channel (ENaC) was identified as a functional
component of the HICC in HepG2 cells. Moreover, it may have a contribution to the basal Na\(^+\) conductance in these cells. Cl\(^-\) conductance was shown to be increased under the \(\alpha\)-ENaC silencing conditions indicating the interplay of cation and anion conductance under hypertonic stress and during RVI.

By means of Scanning Acoustic Microscopy in combination with siRNA the paramount importance of \(\alpha\)-ENaC for RVI process in HepG2 cells could be shown.

Moreover, \(\alpha\)-ENaC protein was shown to contribute to HepG2 cell proliferation. Combination of proliferation assays with cell cycle analysis suggests the requirement of \(\alpha\)-ENaC for the transition of HepG2 cells from G2 into M phase of the cell cycle.

Finally, to identify the proteins interacting with \(\alpha\)-ENaC and to define the overall architecture of the HICC, tagged \(\alpha\)-ENaC was overexpressed in HepG2 cells for the pull down of interacting partners. For the identification of precipitated proteins ESI-MS and Western Blot techniques were employed. It was found, that \(\beta\)-, \(\gamma\)-, and \(\delta\)- subunits of ENaC, as well as TRPM7, TRPP2, ASIC1, and CFTR do very likely not interact with \(\alpha\)-ENaC on the HepG2 cell membrane. Thus, the present study does not give a precise answer concerning the complete identity of the HICC. Further studies a needed to fulfill the identification of all the players contributing to the HICC and thus the RVI process as well as cell proliferation in human hepatocytes.