6 Summary

It was the aim of this work to purify and identify urotensin-II-(UII)-converting enzymes from porcine renal tissue. For this purpose, two systems, the mass-spectrometry-assisted enzyme-screening (MES) and the protein-purification-parameter-system (PPS), were developed and used. MES was used for the detection of UII-generating activity. With PPS parameters for the optimized chromatographic purification of a target protein can be estimated very fast. By applying the MES and the PPS systems nearly homogenous porcine kidney fractions with UII-generating activity were yielded. With the MALDI fingerprint method the pregnancy-associated-glycoprotein (PAG2), and with the LC-ESI MS/MS strategy the disulfide-isomerase-A3 were identified in two different fractions. The catalytic domain of PAG2 is identical with that of pepsin. Since the incubation of the urotensin substrate with pepsin from porcine stomach generates UII as the PAG2 containing fraction, it is likely, that PAG2 is responsible for the UII formation. The disulfide-isomerase-A3 can be easily converted in a serine protease by a simple point mutation. Because the UII-generating fraction was inhibited by aprotinin, a serine protease inhibitor, the propability is high, that an enzyme with a high homology towards the disulfide-isomerase-A3 has an UII-generating activity.

Furthermore, in this work was demonstrated for the first time, that UII may be generated via larger peptides, KPYKKR-UII and KR-UII from its precursor, comparable to angiotensin-II, which is generated from angiotensinogen via angiotensin-I. Future work must prove, if PAG2 and the disulfide-isomerase-A3 are physiologically relevant for the UII-generation.

In conclusion, PAG2, the disulfide-isomerase-A3 and pepsin A were identified, which are able to generate UII. It was shown that UII can be generated stepwise via several precursor-peptides.