

TABLE OF CONTENTS

TABLE OF CONTENTS	1
PREFACE	3
ABBREVIATIONS	4
SUMMARY	6
ZUSAMMENFASSUNG	8
1. INTRODUCTION	10
1.1. Tight junctions.....	10
1.1.1 The morphology and functions of tight junctions.....	10
1.1.2 Model systems to study junction assembly and function.....	13
1.1.3 Molecular components of the tight junction.....	14
1.1.4 Aqueous pores within TJ strands.....	16
1.2. Occludin.....	17
1.2.1 Structure and function of occludin.....	17
1.2.2 Heavily serine/threonine phosphorylated occludin is a component of the TJ.....	20
1.3. Tight junction assembly.....	22
1.3.1 Assembly of the TJ: the role of cadherin mediated cell-cell adhesion.....	22
1.3.2 Assembly of the TJ: the role of protein kinase C.....	24
1.4 Protein kinase C.....	27
1.4.1 Protein kinase C family.....	27
1.4.3 C1-domain proteins: PKC and alternative cellular effectors of DAG and phorbol esters.....	30
1.5 Aims.....	32
2. MATERIALS AND METHODS	33
2.1. Materials.....	33
2.1.1 Chemicals and materials.....	33
2.2. Solutions.....	33
2.2.1 Buffer solutions.....	33
2.3. Methods.....	35
2.3.1. Cell Culture.....	35
2.3.2 Preparation of low and normal calcium medium.....	35
2.3.3 Calcium switch procedures.....	36
2.3.4 Immunofluorescence microscopy.....	36
2.3.5 Sample preparation.....	36
2.3.6 One-dimensional SDS-PAGE and Western blot.....	36
2.3.7 Silver staining of proteins immobilised on nitrocellulose membrane.....	37
2.3.8 RNA isolation.....	37
2.3.9 Quantitation of nucleic acids.....	37
2.3.10 Reverse Transcription-Polymerase chain reaction.....	38
2.3.11 Expression, isolation and purification of GST-fusion proteins.....	38
2.3.12 Occludin in vitro phosphorylation.....	39
2.3.13 Occludin immunoprecipitation and alkaline phosphatase treatment.....	39
2.3.14 Occludin isolation from rat liver.....	39
2.3.15 The determination of the molecular weight and sequence of peptides by mass spectrometry.....	41
2.3.16 Database search.....	42
3.RESULTS	44
3.1. Effect of PKC activators on the phosphorylation and localisation of occludin in MDCK cells cultivated in low calcium medium.....	44
3.1.1 Effects of PKC activators on the TX-100 solubility and phosphorylation of occludin.....	44
3.1.2 Effects of PKC activators on the subcellular localisation of occludin.....	46
3.1.3 Effects of GF-109203X on the phosphorylation and cellular redistribution of occludin induced by PKC activators.....	48
3.2. Effects of PKC inhibitors on phosphorylation and redistribution of occludin induced by Ca ²⁺ Switch.....	49
3.2.1 Effects of GF-109203X and Gö6976.....	49
3.2.2 Gö6976 promotes occludin phosphorylation and tight junction formation.....	51
3.2.3 Rottlerin blocks the tight junction assembly and occludin phosphorylation induced by calcium switch.....	53
3.3 Gö6976 promotes TJ assembly induced by diC8 in LC medium.....	55
3.4. Gö6976 Triggers Tight Junction Formation in MDCK Cells maintained in LC medium.....	57
3.4.1 Effects of Gö6976 on occludin phosphorylation and distribution in LC medium.....	57

3.4.2 Gö6976 Triggers the Formation of Tight Junction Complexes in LC Medium.....	58
3.5. Gö6976 delays tight junction disassembly induced by switch from NC to LC medium.....	60
3.6. Occludin as a Substrate of PKC.....	60
3.6.1 Expression and purification of C-terminal part of occludin.....	60
3.6.2 In vitro phosphorylation of occludin by PKC δ	61
3.7. Identification of the in vivo occludin phosphorylation sites.....	62
3.7.1 Purification of occludin from rat liver.....	62
3.7.2 Mass spectrometric identification of occludin-derived peptides.....	65
3.7.3 Sequencing of the occludin derived peptides by LC-MS/MS.....	69
4. DISCUSSION	73
4.1. PKC-mediated phosphorylation and redistribution of occludin under low calcium conditions.....	73
4.2. Tight junction assembly by calcium switch: the role of conventional PKC.....	77
4.3. Tight junction assembly by calcium switch: the role of novel PKC.....	79
4.4. Gö6976 triggers the formation of tight junction in low calcium conditions and blocks the disassembly of tight junction.....	82
4.5. Conclusion: the assembly of TJs is regulated by the antagonism of conventional and novel protein kinase C isoforms.....	85
4.6. Occludin as a substrate for PKC.....	87
4.7. Purification of occludin from rat liver.....	88
4.8. The identification of <i>in vivo</i> occludin phosphorylation sites.....	91
5. CONCLUSION	96
6. REFERENCE LIST	98