



Contents lists available at ScienceDirect

# Biochimica et Biophysica Acta

journal homepage: [www.elsevier.com/locate/bbamem](http://www.elsevier.com/locate/bbamem)

## Review

# Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery

Mária A. Deli \*

Laboratory of Molecular Neurobiology, Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Temesvári krt. 62., H-6726 Szeged, Hungary

## ARTICLE INFO

### Article history:

Received 28 March 2008

Received in revised form 19 September 2008

Accepted 22 September 2008

Available online 17 October 2008

### Keywords:

Absorption enhancer  
Adherens junction  
Blood–brain barrier  
Drug delivery  
Epithelial cell  
Gastrointestinal barrier  
Nasal drug delivery  
Paracellular transport  
Peptide  
Permeability enhancer  
Tight junction  
Tight junction modulator

## ABSTRACT

The epithelial and endothelial barriers of the human body are major obstacles for drug delivery to the systemic circulation and to organs with unique environment and homeostasis, like the central nervous system. Several transport routes exist in these barriers, which potentially can be exploited for enhancing drug permeability. Beside the transcellular pathways via transporters, adsorptive and receptor-mediated transcytosis, the paracellular flux for cells and molecules is very limited. While lipophilic molecules can diffuse across the cellular plasma membranes, the junctional complexes restrict or completely block the free passage of hydrophilic molecules through the paracellular clefts. Absorption or permeability enhancers developed in the last 40 years for modifying intercellular junctions and paracellular permeability have unspecific mode of action and the effective and toxic doses are very close. Recent advances in barrier research led to the discovery of an increasing number of integral membrane, adaptor, regulator and signalling proteins in tight and adherens junctions. New tight junction modulators are under development, which can directly target tight or adherens junction proteins, the signalling pathways regulating junctional function, or tight junction associated lipid raft microdomains. Modulators acting directly on tight junctions include peptides derived from zonula occludens toxin, or *Clostridium perfringens* enterotoxin, peptides selected by phage display that bind to integral membrane tight junction proteins, and lipid modulators. They can reversibly increase paracellular transport and drug delivery with less toxicity than previous absorption enhancers, and have a potential to be used as pharmaceutical excipients to improve drug delivery across epithelial barriers and the blood–brain barrier.

© 2008 Elsevier B.V. All rights reserved.

**Abbreviations:** 7H6, cytoplasmic tight junction-associated protein; ADT-6, ADT 6-mer peptide corresponding to the bulge in E-cadherin EC-1 domain; AE, absorption enhancer; AJ, adherens junction; AP-1, activator protein 1 transcription factor; BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; BBB, blood–brain barrier; BEC, bronchial epithelial cells; BMVEC, brain microvascular endothelial cell monolayer; Ca<sub>i</sub>, removal of extracellular calcium ions; Ca<sub>i</sub>↑, increase in intracellular calcium ion concentration; CAR, coxsackie and adenovirus receptor; CASK, Ca<sup>2+</sup>-dependent serine protein kinase; CD, cyclodextrin; CNS, central nervous system; CPE, *Clostridium perfringens* enterotoxin; C-CPE, C-terminal fragment of *Clostridium perfringens* enterotoxin; cyclic AMP, cyclic adenosine monophosphate; cyclic GMP, cyclic guanosine monophosphate; DEA-NONOate, diethylammonium (Z)-1-(N,N-diethylamino)diazene-1-ium 1,2-diolate; FSH, follicle stimulating hormone; ΔG, Zonula occludens toxin active fragment (12 kDa); GABA, γ-aminobutyric acid; GAG, glycosaminoglycan; γ-AIB, γ-aminoisobutyric acid; EBA, Evans blue albumin; EC, extracellular; EDTA, ethylenediamine-N,N,N',N'-tetraacetic acid; EGTA, ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid; ERK, extracellular signal-regulated kinase; ESAM, endothelial cell-selective adhesion molecule; FITC, fluorescein isothiocyanate; FSH, follicle-stimulating hormone; GEF-H1, Guanine nucleotide exchange factor H1; GI, gastrointestinal; HAV-6, HAV 6-mer peptide corresponding to the groove in E-cadherin EC-1 domain; His-Zot, Zonula occludens toxin tagged at the N-terminus with a hexahistidine tail; HIV gp120, human immunodeficiency virus 120 kDa glycoprotein; HIV Tat, human immunodeficiency virus transactivator protein; HRP, horseradish peroxidase; ICAM-1, intercellular adhesion molecule-1; Itch, E3 ubiquitin protein ligase; JAM, junctional adhesion molecule; JNK, c-Jun N-terminal kinase; K<sub>Ca</sub>, calcium-activated potassium channel; LPA, lysophosphatidic acid; MAGI 1–3, membrane-associated guanylate guanylate kinase with inverted orientation of protein–protein interaction domains; MAPK, mitogen activated protein kinase; MC-4RA, melanocortin-4 receptor agonist; MBP-Zot, Zonula occludens toxin fused with the maltose-binding protein; MLC, myosin light chain; MLCK, myosin light chain kinase; MRI, magnetic resonance imaging; MUPP 1, multi-PDZ-protein 1; NEC, nasal epithelial cells; NF-κB, nuclear factor-κB transcription factor; NO, nitric oxide; NOC5, 3-(2-hydroxy-1-(methylene)-2-nitrosohydrazino)-1-propanamine; NOC7, (2-hydroxy-1-methylethyl-2-nitrosohydrazino)-N-methyl-1-propanamine; NOC12, N-ethyl-2-(1-ethyl-hydroxy-2-nitrosohydrazino)-ethanamine; NOR1, (+/–)-(E)-4-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide; NOR4, (+/–)-N-[(E)-4-Ethyl-2-[(Z)-hydroxyimino]-5-nitro-3-hexene-1-yl]-3-pyridine carboxamide; OP<sub>90–103</sub>, occludin peptide 90–103; PAPA-NONOate, (Z)-1-[N-(2-aminopropyl)-N-(2-ammonio propyl)amino]diazene-1-ium 1,2-diolate; PAR3/6, partitioning defective proteins 3 and 6; PD, Parkinson's disease; PECAM, platelet-endothelial cell adhesion molecule; PEG, polyethylene glycol; PEG 4000, polyethylene glycol 4 kDa; Pgp, P-glycoprotein; PGPC, 1-1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine; PI3, phosphatidylinositol 3,4,5-triphosphate; PI3K, phosphoinositide 3-kinase; PI3K/Akt, phosphoinositide 3-kinase/Akt pathway; PKA, protein kinase A; PKG, protein kinase G; PKC, protein kinase C; PLC, phospholipase C; PPE, paracellular permeability enhancer; Rac, a small GTPase of the Rho family; REC, respiratory epithelial cells; RGS5, regulator of G-protein signalling 5; Rho/ROCK, Rho/Rho-associated protein kinase pathway; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; siRNA, small interfering ribonucleic acid; SNAP, S-nitroso-N-acetyl-DL-penicillamine; TCDC, taurochenodeoxycholate; TEC, tracheal epithelial cells; TEER, transepithelial/transendothelial electrical resistance; TJ, tight junction; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor; ZO-1, zonula occludens protein 1; ZONAB, ZO-1-associated nucleic acid-binding protein; Zot, zonula occludens toxin of *Vibrio cholerae*

\* Tel.: +36 62 599 602; fax: +36 62 433 133.

E-mail address: [deli@brc.hu](mailto:deli@brc.hu).

## Contents

1.	Introduction . . . . .	893
2.	Major epithelial and endothelial barriers and drug delivery . . . . .	893
2.1.	Epithelial barriers of the gastrointestinal tract . . . . .	893
2.2.	Airway epithelium. . . . .	894
2.3.	Skin . . . . .	894
2.4.	Blood–brain barrier . . . . .	894
2.5.	Alternative routes for drug delivery . . . . .	894
3.	Potential targets of TJ modulators . . . . .	894
3.1.	Transmembrane TJ proteins . . . . .	894
3.2.	Transmembrane adherens junction proteins as targets of paracellular barrier modulators . . . . .	895
3.3.	Signalling pathways regulating TJ functions . . . . .	896
3.4.	Lipid rafts and membrane microdomains . . . . .	897
4.	Modulators acting directly on TJ components . . . . .	897
4.1.	Occludin . . . . .	897
4.2.	Claudins . . . . .	899
4.3.	Zonulin . . . . .	899
4.4.	E-cadherin . . . . .	899
4.5.	Other targets . . . . .	899
5.	TJ modulating effects of absorption enhancers/paracellular permeability enhancers . . . . .	899
5.1.	Chelators . . . . .	901
5.2.	Fatty acids, modified fatty acids and phosphate esters . . . . .	902
5.3.	Surfactants . . . . .	902
5.4.	Cationic polymers . . . . .	902
5.5.	Cyclodextrins . . . . .	902
5.6.	Nitric oxide donors . . . . .	902
6.	Modulation of TJs at the blood–brain barrier for drug targeting . . . . .	903
6.1.	Modulators acting on brain endothelial TJs . . . . .	903
6.2.	Permeability enhancers and new experimental approaches . . . . .	904
7.	Concluding remarks . . . . .	905
	Acknowledgements . . . . .	905
	References . . . . .	905

## 1. Introduction

Epithelial and endothelial barriers separate the organisms from the external environment and the body compartments from each other. The tight intercellular seals responsible for the effective separation were identified as zonula occludens or tight junctions (TJ) by electron microscopy more than 50 years ago (for review see [1]). In the last 25 years the discovery of the transmembrane proteins of TJs [2] and the signalling pathways regulating TJ functions [3] resulted in a great advance in understanding how epithelial and endothelial barriers work and how this knowledge can be exploited in disease therapies, drug discovery and drug targeting.

The non-invasive delivery of hydrophilic drugs or large biopharmaceuticals to the systemic circulation or to specific organs protected by a barrier, like the central nervous system (CNS), is still a big challenge. Drug absorption or penetration across epithelial and endothelial barriers is restricted by several factors. Efflux transporters expressed at high levels in epithelia [4] and the blood–brain barrier (BBB) [5] prevent not only the flux of xenobiotics, but also drug molecules to reach their target. Local metabolic barriers act as a second line of defence and specific enzymes are able to inactivate drugs, cleave proteins and peptides, or other biologically active compounds [6]. The third major cause is the presence of intercellular TJs that strictly limit the paracellular route. In the last 50 years several methods, molecules, and excipients have been investigated for the safe and reversible opening of these junctions to enhance drug absorption and penetration. Low efficacy and high toxicity prevented these approaches to be useful for pharmaceutical therapy. New TJ modulators, designed to interact directly with TJ proteins or regulating molecules, are promising candidates to improve drug delivery.

## 2. Major epithelial and endothelial barriers and drug delivery

The paracellular permeability, one of the most important determinants of drug transport, of various epithelial tissues and barriers differs greatly [7]. Transepithelial electrical resistance (TEER), measuring paracellular ion flux and reflecting the tightness of the intercellular junctional complex, is the lowest in the small intestine, which is considered as a leaky epithelial tissue. The TJs in the colon and the stomach are of intermediate tightness, while brain capillaries and the skin epithelial cells form very tight paracellular barriers.

### 2.1. Epithelial barriers of the gastrointestinal tract

Oral administration of drugs is the most popular and widespread method of drug therapy. The patients' compliance is high, because it is safe, efficient, easily accessible and causes minimal discomfort. Membrane permeability is one of the major factors which determine drug absorption after oral administration. The total surface area of the intestine is approximately 200 m<sup>2</sup>. Except for the buccal and rectal mucosa, where the surface consists of stratified squamous epithelium, a columnar epithelial cell layer covers the surface in the other parts of the gastrointestinal (GI) tract. The lower parts of the small intestine, jejunum and ileum, are considered as the major place of drug absorption, because of the leaky paracellular tight junctions reflected by the low transepithelial electrical resistance, as compared to the other parts of the GI tract [7]. The villous structure of the jejunum and ileum amplifies the surface area four and two-folds, respectively, as compared to the colon [8], another factor in drug absorbance. Although the gastric epithelium is much tighter than the small intestine [7], gastric absorption can be substantial for drugs administered in rapidly dissolving formulations or for lipophilic

molecules [8]. The great differences in the paracellular permeability between the regions of the GI tract [8] are caused by the dissimilar anatomical structures, distinct lipid composition of the plasma membranes and expression of diverse members of the claudin TJ protein family [2].

The paracellular permeability pathway in the buccal and rectal mucosa is restricted, and their surface area is small (approx. 100–200 cm<sup>2</sup>), nevertheless they are important drug delivery sites. Due to their special anatomical location the blood from their submucosa is directly drained toward the superior or inferior vena cava, respectively, avoiding first pass metabolism in the liver and intestine, which is important for a fast effect in crisis therapy (e.g. anti-angina treatment, anticonvulsants, analgesics, antipyretics).

## 2.2. Airway epithelium

Drug delivery across pulmonary epithelia, ciliated epithelial cells, type I and type II pneumocytes, is an attractive route for non-invasive local and systemic therapy [9]. The alveolar surface area is large, about 160 m<sup>2</sup>, close to the surface area of the GI tract. Pulmonary drug delivery systems for macromolecules, peptides and proteins, like insulin, antibodies and vaccines have been developed and their applications have a great therapeutical potential [9]. Exubera, the first inhaled insulin was approved for clinical use in Europe and the United States, but marketing was stopped in 2007.

## 2.3. Skin

From all of the epithelial barriers the skin is the least permeable to molecules due to its special anatomical and molecular structure. The stratum corneum, the outermost layer composed of keratin-filled corneocytes in a special lipid matrix, is responsible for the extremely low permeability to water and solutes. Despite the fact, that skin represents a major barrier to drug transport, transdermal drug delivery is the most successful non-oral technology for systemic drug delivery in the pharmaceutical market [10]. Transdermal systems offer a non-invasive, slow, sustained drug delivery across an accessible and relatively large, 1–2 m<sup>2</sup> surface area [10].

## 2.4. Blood–brain barrier

The BBB, a dynamic interface separating the brain from systemic circulation, is the major entry route for therapeutical compounds to the CNS. The estimated total length of human brain capillaries is 650 km, with a total surface area of 10 to 20 m<sup>2</sup> [11]. The complex tight junctions between brain endothelial cells constitute the morphological basis of the BBB [12]. The primary role of the BBB is to create ionic homeostasis for neuronal functions [13]. It also provides the CNS with nutrients and protects it from toxic insults by sophisticated transport systems [5]. The low level of paracellular flux and transendothelial vesicular trafficking result in a transport barrier for drugs which are hydrophilic and have a molecular mass bigger than 400 Da, while the presence of effective efflux transporters at the luminal membrane of brain endothelial cells limits the brain penetration of lipophilic xenobiotics and drugs. The BBB prevents 98% of potential neuropharmaceuticals, especially the new peptide or protein drugs, to reach their targets in the CNS [14]. Due to these reasons the treatment of CNS diseases, including stroke, Alzheimer's disease, and brain tumours, is still unsatisfactory and improving drug delivery to the CNS is considered essential for the future success of the therapy of neurological disorders [15].

## 2.5. Alternative routes for drug delivery

Besides conventional local treatments, the nasal route can be also exploited for systemic, non-invasive delivery of drugs [16]. The nasal

epithelial surface area is small, about 150 cm<sup>2</sup>, and consists of respiratory and olfactory regions. Although the olfactory system possesses elaborate epithelial, endothelial and glial barriers expressing occludin, claudins and other TJ proteins [17], due to its special anatomical localization it provides a direct access to the CNS by circumventing the BBB [18]. Another advantage of the nasal delivery is the rapid onset of action, which makes this route suitable for crisis treatments. An increasing number of nasal formulations are used clinically for the treatment of migraine [16].

A new alternative route to brain across the epithelial barriers of the eye emerges, which also bypasses the BBB. Recent experimental results indicate the possibility of drug delivery to the CNS following ocular application through non-systemic routes [19,20].

## 3. Potential targets of TJ modulators

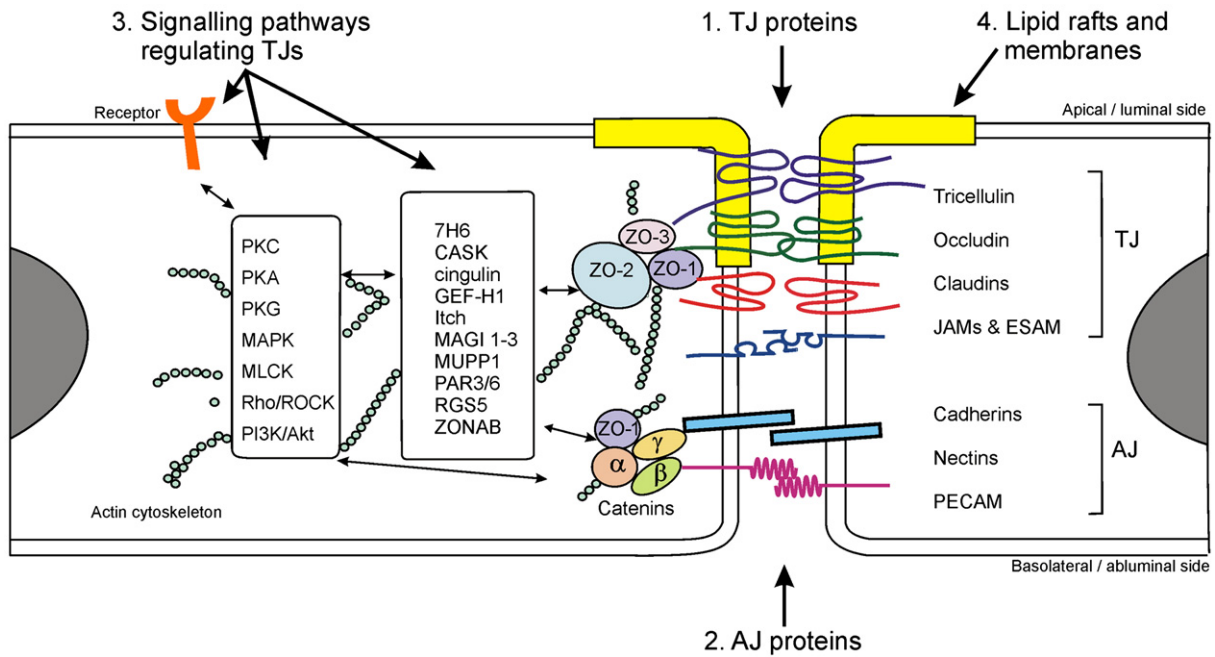
Tight junctions, sealing the paracellular clefts between cells of the epithelial and endothelial barriers in mammals are elaborate structures composed of integral membrane proteins (for reviews see [2,21]), linker or adaptor proteins connecting them to the actin cytoskeleton [22] and signalling molecules enabling the dynamic regulation of the paracellular transport [3]. Adherens junctions (AJ) are also present in both types of barriers, however differences in their localization, morphology, and molecular composition exist. While in brain endothelial cells tight and adherens junctions are intermingled [23], in epithelial barriers AJs are situated in the apical junctional complex in basolateral direction from the TJs. Some of the junctional proteins are found in raft-like membrane microdomains in epithelial cells [24]. These compartments can play an important role in the spatial organization of TJs and probably in the regulation of epithelial paracellular permeability. Theoretically all these components listed above can be considered as potential targets of TJ modulators (Fig. 1). Some of the junctional proteins seem to be excellent targets for safe modulation of paracellular permeability, while targeting other adhesion molecules playing a fundamental role in morphogenesis should be considered cautiously, e.g. where loss of function is strongly linked to carcinogenesis.

### 3.1. Transmembrane TJ proteins

Occludin, the first integral membrane TJ protein, was supposed to contribute to both the gate and fence functions [25]. Experiments with occludin-deficient cells and animals revealed morphologically unaffected TJs and no barrier dysfunction [2]. However, chronic inflammation and hyperplasia of the gastric epithelium, calcification in the brain, testicular atrophy and other histological abnormalities in the salivary gland and in the bones were observed in occludin deficient mice [26] suggesting more complex functions of occludin. Occludin internalization by caveolar endocytosis was found to be linked to barrier dysfunction in intestinal inflammation (for review see [27]). From the accumulated data a new role for occludin as a signal transmitter in TJs regulating development, apoptosis in healthy and cancer cells, and actin cytoskeleton emerges [2].

Tricellulin, a recently discovered TJ protein showing partial homology with occludin, is expressed uniquely at tricellular contacts of epithelial cells [28]. It also participates in the barrier function and organization of bicellular junctions. Interestingly, the only described phenotype of tricellulin gene mutation is deafness, due to the loss of the protein from tricellular junctions in cochlear and vestibular epithelial cells [29].

Claudins form a large gene family with 24 members identified so far, which are able to determine the tissue-, charge- and size-selectivity of the paracellular seals [2,21]. Data from different claudin null mutant mice strains and transfection experiments indicate that claudin-1, -3, -4, -5, -8, -11, -14 and -19 contribute to the tightness of paracellular barriers [21]. Claudins are key elements in the loss of



**Fig. 1.** Schematic model of targets of tight junction modulators in epithelial and endothelial cells. *Abbreviations:* 7H6, cytoplasmic tight junction-associated protein; AJ, adherens junction; CASK,  $\text{Ca}^{2+}$ -dependent serine protein kinase; ESAM, endothelial selective adhesion molecule; GEF-H1, Guanine nucleotide exchange factor H1; Itch, E3 ubiquitin protein ligase; JAMs, junctional adhesion molecules; MAGI 1–3, membrane-associated guanylate guanylate kinase with inverted orientation of protein–protein interaction domains; MAPK, mitogen-activated protein kinase; MLCK, myosin light chain kinase; MUPP 1, multi-PDZ-protein 1; PAR3/6, partitioning defective proteins 3 and 6; PECAM, platelet-endothelial cell adhesion molecule; PI3K/Akt, phosphoinositide 3-kinase/Akt pathway; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; RGS5, regulator of G-protein signalling 5; Rho/ROCK, Rho/Rho-associated protein kinase pathway; TJ, tight junction; ZO-1, zonula occludens 1; ZO-2, zonula occludens 2; ZO-3, zonula occludens 3; ZONAB, ZO-1-associated nucleic acid-binding protein.

barrier function during carcinogenesis and cancer metastasis (for review see [30]).

The paracellular ion permeability through TJs is regulated by both sealing and pore forming claudins. Claudin-4, -8, and -14 function primarily in cation barriers, while other members of the claudin family, claudin-2, -7, -10, -13, -15, -16, form paracellular pores for cations or anions [2]. They also participate in magnesium and calcium reabsorption, as revealed by the mutation of the human claudin-16 and 19 genes [31,32]. In MDCK mammalian renal epithelial cells tight junction leakiness is conferred by expression of claudin-2 [33].

The differential expression of claudins in tissues and cell types provide the basis for the large spectrum of the paracellular tightness of the junctional complexes in various organs [2]. The epidermal barrier is disrupted in claudin-1 deficient mice [34], and claudin-11 plays a role in the blood–testis barrier [35]. In the GI tract claudin-2, -3, -4, -7, -8, -12 and -15 are all expressed, but the level of their expression in various intestinal segments and their subcellular localization are different [2]. Their role in the structural organization of the intestinal barrier is emphasized by a recent finding. Claudin-15 deficient mice show a megaintestine phenotype, the upper part of the small intestine is two times larger than normal in length and diameter [36].

The presence of claudin-1, -3, -5, -12 and -18 were described in brain endothelial cells [21]. A special regulatory role for claudin-5, expressed in endothelial but not in epithelial cells, was discovered in a knock-out mice. Tracer experiments and magnetic resonance imaging revealed that in the absence of claudin-5 the BBB permeability was selectively increased for small molecules (<800 Da), but not for larger molecules [37].

Junctional adhesion molecules (JAMs), another family of integral membrane proteins in TJs, are involved in intercellular adhesion between the cells of barriers, as well as in adhesion between barriers and blood cells [2,22]. JAM-A, expressed in both epithelial and endothelial cells, and coxsackie and adenovirus receptor (CAR)

expressed only in epithelial cells, contribute to barrier function and cell polarity in epithelial and endothelial cells, while JAM-B and -C participates in spermatogenesis [2]. In addition, JAM-A and JAM-C control transmigration of neutrophils and monocytes across endothelial and epithelial cells, another important aspect of barrier functions. Endothelial cell-selective adhesion molecule (ESAM) is expressed in vascular endothelial cells, where it colocalizes with cadherins and catenins in junctions [38]. ESAM is present at the BBB and it is enriched in the BBB genome [39]. Loss of ESAM alters angiogenesis in pathological conditions, reduces extravasation of neutrophil leukocytes and vascular endothelial growth factor (VEGF)-induced permeability in vessels [40].

Integral membrane TJ proteins also serve as receptors for pathogenic bacteria and viruses, and they can be the targets of bacterial toxins (for review see [41]). Claudin-1 is a co-receptor for hepatitis virus C, and claudin-4 is a receptor for *Clostridium perfringens* enterotoxin (CPE), while JAM-A binds reoviruses, and coxsackie and adenoviruses target CAR [2]. CPE served as a model sequence for the design of new, highly specific TJ modulator peptides [42,43].

### 3.2. Transmembrane adherens junction proteins as targets of paracellular barrier modulators

AJs, present in both epithelial and endothelial barriers initiate and stabilize cell–cell contacts and help the formation of TJs [44]. AJ proteins also participate in the regulation of barrier permeability.

Similarly to JAMs, the calcium-dependent adhesion molecules, cadherins also belong to the immunoglobulin superfamily. E-cadherin is present in all epithelial barriers, and its presence was also demonstrated in brain endothelial cells [45]. E-cadherin is important for establishing and maintaining apico-basal polarity as well as for the formation of TJs [46]. Loss of E-cadherin in the epidermis, by conditionally inactivating its gene in mice, results in perinatal death due to the inability to retain a functional epidermal water barrier [47].



Loss of E-cadherin increases TJ permeability, and alters the localization of claudin-1, -4 and ZO-1 at TJs through activation of Rac, a small GTPase of the Rho family, and protein kinase C (PKC) [47]. Histamine increases airway epithelial cell permeability by decreasing E-cadherin-based epithelial cell adhesion [48]. E-cadherin plays a basic role in morphogenesis. Embryonic stem cells isolated from E-cadherin<sup>-/-</sup> preimplantation embryos fail to differentiate into any organized structure when induced to form teratomas in mice [46]. The loss or down-regulation of E-cadherin is a key event in the process of cancer invasion and metastasis [49]. Mutations in *CDH1* that compromise the adhesive function of E-cadherin have been observed in human gastric carcinoma cell lines, lobular breast cancer, and familial gastric cancer [49].

Vascular endothelial, or VE-cadherin, another member of the large cell adhesion molecule cadherin family, is highly and uniquely expressed in the endothelium of all types of vessels [50]. VE-cadherin is indispensable for the developmental organization of the vasculature, and its inactivation or truncation leads to an embryonic lethal phenotype [50]. Vasoactive and proinflammatory mediators disrupt endothelial adherens junctions, especially the association of VE-cadherin with the cytoplasmic linker molecules catenins resulting in an increase in vascular permeability [51]. It has been recently identified, that VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin [52].

The nectins form another important family of adhesion molecules in AJs [53]. These immunoglobulin-like cell adhesion molecules provide the first scaffold for AJ and TJ formation. Nectins-1, -2, -3 are expressed in epithelial, endothelial, hematopoietic and neuronal cells in adult tissues, while nectin-4 is mainly expressed during embryogenesis [54]. Nectin-4 has been identified as a new histological and serological tumour associated marker for breast cancer [55].

Platelet-endothelial cell adhesion molecule-1 (PECAM-1) is present in endothelial and blood cells, but absent in epithelial barriers [50]. It participates in the formation of endothelial junctions and in the transmigration of leukocytes across endothelium [56]. Effective treatment of gut barrier dysfunction was achieved using monoclonal antibodies against PECAM-1 [57]. A new role for PECAM-1 is suggested at the BBB. By counteracting intercellular adhesion molecule-1 (ICAM-1)-induced activation in brain endothelial cells it can limit rearrangements of the actin cytoskeleton and maintain the integrity of brain vascular endothelium [58].

Similarly to transmembrane TJ proteins, some of the AJ proteins can bind infectious agents. E-cadherin, transiently exposed at sites of cell extrusion, is a specific target of *Listeria monocytogenes* adhesion and invasion by binding Internalin A, bacterial cell surface protein [59]. Nectin-1 is a cell-surface receptor for herpes simplex virus type 1 envelope glycoprotein D, and it is used for targeting attenuated, oncolytic herpes simplex virus to infect and lyse human malignancies in preclinical studies [60].

### 3.3. Signalling pathways regulating TJ functions

The dynamic changes at the junctions, disintegration and reassembly of TJs, reversible changes in paracellular permeability, are regulated by signalling pathways in epithelial barriers and the BBB (for reviews see [3,61,62]). The major signalling routes that participate in TJ opening with a potential to be exploited for drug delivery involve protein kinases A, C and G (PKA, PKC, PKG), Rho kinases, myosin light chain kinase (MLCK), and the mitogen activated protein kinase (MAPK) system (Fig. 1) [3,61,62]. The effects of certain pathways greatly differ between tissues, cell types, and models, so it is difficult to draw general conclusions.

Conventional PKC isoforms ( $\alpha$ ,  $\beta$  II) participate in TJ opening or disassembly [61]. A wide range of pathological and physiological signals acts through PKC, like removal of extracellular  $\text{Ca}^{2+}$ , elevation

of intracellular  $\text{Ca}^{2+}$  levels by phorbol esters or  $\text{Ca}^{2+}$  ionophore A23187 [3,62].  $\text{Ca}^{2+}$  chelators, used as absorption enhancers (AE) at different barriers also activate PKC. Pathological stimuli, like oxidative stress, cytokines (tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), etc.), and vascular endothelial growth factor (VEGF), as well as toxins from infectious agents, HIV-1 gp120, *Vibrio cholerae* zonula occludens toxin (Zot), *Clostridium difficile* toxin A, *Escherichia coli* OmpA all use PKC signalling to open tight junctions [3]. Bryostatin 1, a novel anticancer agent and a non-phorbol ester stimulator of PKC transiently increases TJ permeability in epithelial cells through rapid downregulation of PKC- $\alpha$  [63].

While the role of PKA is controversial in TJ regulation in epithelial barriers, it is linked to barrier tightening in brain endothelial cells [3,62]. Elevation of intracellular cyclic AMP by cyclic-AMP analogues [64], phosphodiesterase 4 inhibitors, or by physiological regulators, like the peptide hormone adrenomedullin [65] results in increased TEER and decreased paracellular permeability [62,66] through enhanced expression of claudin-5 at TJs [67]. Inhibitors of this pathway could increase the paracellular permeability at the BBB.

In contrast, PKG mediates BBB opening via soluble guanylate cyclase activation and elevation of cyclic GMP [3,62,66]. The bradykinin B2 receptor agonist Cereport acting through this pathway could effectively and reversibly open brain endothelial TJs [68]. Pathological conditions, like hypoxia, ischemia or excessive nitric oxide (NO) release, either endogenous, or from NO-donors, also activate soluble guanylate cyclase and PKG and increase BBB permeability [62,66].

One of the main effector mechanisms in TJ regulation is the phosphorylation of myosin light chain (MLC) by MLCK leading to the contraction of the acto-myosin belt and disassembly of TJs [61]. Cytokines, bacterial or viral pathogens, removal of extracellular  $\text{Ca}^{2+}$ , as well as bile acids, used as absorption enhancers (AEs), trigger TJ opening via MLCK activation [3]. The vasoactive mediator histamine and the lysophosphatidic acid (LPA) also compromise the integrity of brain endothelial cells by MLCK activation [69].

Mitogen activated protein kinases (MAPKs) modulate paracellular permeability of TJs by regulating the expression of several TJ proteins (for review see [3,61]). Three of the four groups of MAPKs, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 isoforms participate in regulation of the barrier function. Ethanol-induced decrease in the barrier function uses all three pathways, while proinflammatory cytokines can induce both Erk1/2 and p38 MAPKs. Bile salts, reactive oxygen species (ROS), pathogenic factors of viruses and bacteria activate predominantly the Erk1/2 via Ras and Raf [3]. While all the above mentioned pathways result in a decrease in the paracellular barrier function, activation of Erk1/2 signalling pathway by negatively controlling claudin-2 expression can also enhance paracellular tightness in MDCK renal epithelial cells [33]. In contrast, Erk1/2 inhibition by inducing claudin-2 expression in renal epithelial cells leads to a decreased TEER [33], which is in agreement with the role of claudin-2 known to be able to form paracellular pores for cations and anions [2]. This observation indicates that regulation of paracellular barrier tightness, at least for ions, can be achieved by alteration in claudin composition within TJ complexes. Furthermore, the polymerization of claudins is critically dependent on binding to TJ membrane-associated guanylate kinase proteins ZO-1, ZO-2 and ZO-3. In Eph4 mouse epithelial cells with no expression of ZO proteins a complete lack of TJs could be observed [70].

Activation of RhoA, Rac1 and Cdc42, members of the Rho family of GTPases, all disrupt epithelial tight junctions [71]. RhoA interacts with the PKC and MLCK pathways and regulates TJ disassembly through phosphorylation of integral membrane TJ proteins occludin and claudin-5 and scaffolding proteins ZO-1 and ZO-2 [3]. The level of RhoA is critical for TJ regulation, and either inactivation of it by C3 transferase, a specific inhibitor, or activation by cytokine interferon- $\gamma$

and 2-methoxyestradiol, a microtubule destabilizing drug, leads to TJ disassembly [3]. RhoA participates in reduced brain endothelial TJ functions after LPA treatment [69], and in ROS-induced alterations in BBB integrity and monocyte migration across brain endothelial cells [72].

The activation of phosphoinositide 3-kinases (PI3Ks) can induce both tightening and opening of TJs depending on the inducing agent and barrier type [61]. While the PI3K/Akt pathway participates in the restitution of injured barrier in intestinal epithelium [73], the same PI3-K/Akt signalling route is responsible for hyperpermeability induced by ROS [72,74], placenta-derived growth factor, VEGF [75], HIV gp120 [76] and HIV Tat [77] in BBB models.

### 3.4. Lipid rafts and membrane microdomains

It is known for a long time, that lipids are involved in the formation and regulation of TJs and lipid models of the TJs have been suggested to explain lipid exchange between the apical membranes of neighbouring the cells, i.e. the “bridge” function of cell–cell adhesions (Fig. 1) [78]. TJs are enriched in cholesterol [79] and alterations in cell cholesterol modulate junctional permeability for ions [80]. TJ proteins occludin, claudin-1, -3, -4, -7, JAM-A and ZO-1 have been shown to be associated with detergent insoluble glycolipid raft structures rich in caveolin-1 [24,81]. TJ disassembly by calcium chelation resulted in displacement of TJ proteins from the lipid rafts suggesting an important role for these structures in regulation of paracellular permeability [24]. Data indicate that alteration in lipid composition of epithelial cell membrane, especially cholesterol efflux, can result in TJ modulation [81,82]. Depletion of cholesterol from Caco2 epithelial

cells by methyl- $\beta$ -cyclodextrin, used as AE, results in loss of TJ integrity and displacement of occludin, claudin-3, -4, -7 and JAM-A out of the cholesterol rich membrane domains [81]. Lipid mediators and second messengers activating signalling pathways that regulate TJ permeability can mediate the effect of cholesterol depleting agents. These data suggest that TJ modulation can be achieved by changing the lipid composition of the cellular membranes or TJ-associated lipid rafts of barrier forming cells (Fig. 1).

## 4. Modulators acting directly on TJ components

Better understanding the molecular mechanisms that regulate the paracellular pathway led to the discovery of novel TJ modulators that directly and specifically interact with the extracellular loops of TJ proteins and the TJ-associated membrane microdomains [43,83–85]. These peptides, enzymes or lipids can be potentially used as new excipients to safely and reversibly open TJs for delivery of hydrophilic compounds and biopharmaceuticals (Table 1).

### 4.1. Occludin

Occludin is the first TJ protein, where modulation of TJ permeability by a peptide interacting with its extracellular loop was described [86]. The 44 amino acid long OCC2 peptide (Table 2), binding to the second extracellular domain of occludin induced a reversible increase in paracellular permeability by an increased turnover of cellular occludin in kidney epithelial cells [86]. The effect was specific, because ZO-1, ZO-2, cingulin, and E-cadherin levels and distribution were unaltered by OCC2 treatment. The same

**Table 1**

Targets and mode of action of modulators directly acting on tight junction components

Target molecule	Mechanism	Modulator substance	Reference
<i>Modulators acting on tight junction proteins</i>			
<i>Occludin</i>			
Occludin	EC loop analogue	Synthetic peptide	[86]
	EC loop analogue	9–10 amino acid peptides	[87]
	EC loop analogue	14 amino acid lipopeptide OP <sub>90–103</sub>	[88,89]
	Ligand-fused occluding	$\Delta$ FSH-fused occludin peptide	[90]
	Peptide analogues	4–25 contiguous amino acid peptides	[91]
	Cleavage of first EC loop	Der P1 cysteine proteinase	[92]
	Gene expression	Antisense oligonucleotide	[93]
	Gene expression	siRNA	[94]
<i>Claudins</i>			
Claudin-4	CPE receptor	C-terminal region of CPE	[95]
Claudin-4	Gene expression	siRNA	[94]
Claudins 1–10	Peptide analogues	4–25 contiguous amino acid peptides	[91]
Claudin-23	EC loop of claudin-23	8–20 contiguous amino acid peptides	[96]
<i>Junctional adhesion molecules</i>			
JAMs 1–3 peptide analogues 425 contiguous amino acid peptides [91]	Peptide analogues	4–25 contiguous amino acid peptides	[91]
JAM-1	Gene expression	siRNA	[94]
<i>Modulators acting on adherens junction proteins</i>			
E-cadherin			
E-cadherin	EC1 domain analogue	HAV 6-mer peptide	[97]
	EC1 domain analogue	ADT 6-mer peptide	[97]
<i>Modulators acting on signalling pathways regulating tight junctions</i>			
Zot receptor	PKC- $\alpha$ activation	<i>Vibrio cholerae</i> Zot (45 kDa)	[98–100]
Zot receptor	PKC- $\alpha$ activation	$\Delta$ G (Zot active fragment; 12 kDa)	[101]
Zot receptor	PKC- $\alpha$ activation	Zonulin	[102]
Zot receptor	PKC- $\alpha$ activation	AT-1002	[103]
<i>Modulators acting on lipid rafts and membranes</i>			
Lipid rafts	TJ/AJ dissociation	PGPC and glycerol-3-phosphocholines	[85]
<i>Modulators acting on miscellaneous targets</i>			
[Not disclosed]	Peptide analogues	PN peptides	[104,105]
TJ GAGs	GAG-degrading enzymes	Heparinases, chondroitinases	[106]

Abbreviations: AJ, adherens junction; CPE, *Clostridium perfringens* enterotoxin; EC, extracellular; FSH, follicle stimulating hormone; GAG, glycosaminoglycan; JAM, junctional adhesion molecule; OP<sub>90–103</sub>, occludin peptide 90–103; PGPC, 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine; PKC, protein kinase C; siRNA, small interfering ribonucleic acid; TJ, tight junction; Zot, zonula occludens toxin.

**Table 2**

Amino acid sequence of selected peptide tight junction modulators

Peptide TJ modulator	Amino acid sequence	Reference
<i>Occludin</i>		
Occludin 44-mer peptide	184 GVNPQAQMSSGYYSPLAMCSQAYGSTYLNQYIYHYCTVDPQE	[86]
Occludin 22-mer peptide	209 NH <sub>2</sub> -GSQJYTICSQFYTPGGTGLYVD-COOH	[82,90]
Lipopeptide OP <sub>90–103</sub>	H <sub>2</sub> N-CHR-CONH-DRGYGTSLLGGSVG	[88,89]
Occludin 10-mer peptide	100 SNYYGSGLSY	[87]
Occludin 9-mer peptide	100 SNYYGSGLS	[87]
7-mer peptide	FDFWITP	[107]
Occludin 6-mer peptide B (cyclic)	H-CLYHYC-OH	[108]
<i>Claudin-4</i>		
C-CPE	1 DIEKEILDLA AATERLNLTDL ALNSNPAGNL YDWRSSNSYP WTQKLNHLTL 51 ITATGQKYRI LASKIVDFNI YSNNFNLLVK LEQSLGDGVK DHYVDISLDA 101 GQYVLMKAN SSYSGNYPYS ILFQKF	[95]
C-CPE C-terminal 30-mer	NH <sub>2</sub> -SLDAGQYVLV MIKANSYSYGN YPYSILFQKF-OH	[109]
C-CPE C-terminal 16-mer	NH <sub>2</sub> -SSYSGNYPYS ILFQKF-OH	[110]
<i>E-cadherin</i>		
E-cadherin 6-mer HAV-6	Ac-SHAVSS-NH <sub>2</sub>	[111]
E-cadherin 6-mer ADT-6	Ac-ADTPPV-NH <sub>2</sub>	[111]
<i>Zonula occludens toxin</i>		
<i>Vibrio cholerae</i> Zot; 45 kDa (zonula occludens toxin)	1 MSIFIHHGAP GSYKTSGALW LRLLPKAKSG RHIITNVRGI NLERMAKYLK 51 MDVSDISIEF IDTDHPDGRI TMAFWHWHAR KDAFLFIDEC GRIWPPRITA 101 TNLKALDTPP DLVAEDRPES FEVAFDMHRH HGWDICLTPP NIAKVHNMIR 151 EAAEIGYRHF NRATVGLGAK FTITTHDAAN SQQMDSHALT RQVKKIPSI 201 FKMYASTTTG KARDTMAGTA LWKDRKILFL FGMVFLMFSY SFYGLHDNPI 251 FTGGNDATIE SEQSEPSKA TAGNAVGSKA VAPASFGFCI GRLCVQDGFV 301 TVGDERYRLV DNLDLPYRGL WATGHHLYKD KLTVFFETES GSVPTLFFAS 351 SYRYKVLPLP DFNHFVVDFT FAAQALWVEV KRGLPLKTEN DKKGINSIF	[112]
ΔG; 12 kDa (Zot active fragment)	265 EPQSKA TAGNAVGSKA VAPASFGFCI GRLCVQDGFV 301 TVGDERYRLV DNLDLPYRGL WATGHHLYKD KLTVFFETES GSVPTLFFAS 351 SYRYKVLPLP DFNHFVVDFT FAAQALWVEV KRGLPLKTEN DKKGINSIF	[112]
AT-1001 (FZI/0 synthetic inhibitor)	GGVLVQPG	[103,113]
AT-1002 (Zot active domain)	FCIGRL	[112]
<i>Not disclosed target</i>		
PN 159	NH <sub>2</sub> -KLALKLALKALKAAKLKLA-amide	[104,105]
PN 393 (all D-substituted)	NH <sub>2</sub> -klalklalkalkaalkla-amide	[104]
PN 407	NH <sub>2</sub> -LKILKkLlKLLkLL-amide	[104]
PN 425	NH <sub>2</sub> -KLAWKLALKALKAAKLKLA-amide	[104]
PN 427	NH <sub>2</sub> -KLAWKLALKALKAAWKLKLA-amide	[104]
PN 679	CNGRCGGKKKLLKLLKLL	[105]
PN 745	LRKLRLRLRLRLKRLRLR-amide	[105]

Abbreviations: ADT-6, ADT 6-mer peptide corresponding to the bulge in E-cadherin EC-1 domain; C-CPE, C-terminal peptide of *Clostridium perfringens* enterotoxin; HAV-6, HAV 6-mer peptide corresponding to the groove in E-cadherin EC-1 domain; OP<sub>90–103</sub>, occludin peptide 90–103; TJ, tight junction; Zot, zonula occludens toxin.

peptide also decreased TEER and depleted occludin from TJs in EpH4 mouse mammary epithelial cell line, but induced the formation of unpolarized multi-layered cell clusters [114]. Parallely, upregulation and translocation of AJ protein  $\beta$ -catenin was induced, and through  $\beta$ -catenin/TCF/LEF activation led to the upregulation of the protooncogene *c-myc* [114].

Oligopeptides (9-mer and 10-mer) homologous to segments of the first external loop of occludin impaired junctional resealing on cultured epithelial cells in a reversible manner without altering ZO-1 localization [87].

A 14 amino acid long peptide, OP<sub>90–103</sub>, corresponding to the N terminus of the first extracellular loop of occludin and containing a lipoamino acid to protect it from degradation and aggregation increased TJ permeability in Caco-2 cells treated apically [88]. The effect of OP<sub>90–103</sub> was rapid, transient, and caused no short-term toxicity. The peptide reduced TEER and increased 70 kDa dextran transport in a concentration-dependent manner in human primary airway epithelial cells [89] (Table 3). Treatment with OP<sub>90–103</sub> peptide resulted in enhanced gene transfer efficiency of epithelial cells with adenovirus vectors due to TJ opening, increased penetration to the basolateral membrane, and greater viral binding and internalization [89].

Organ-specific targeting of a 22 amino acid long peptide interacting with the second extracellular loop of occludin [86] (Tables 1 and 2)

has been demonstrated in the blood–testis barrier in rats [90] (Tables 1–3). A genetically engineered follicle-stimulating hormone (FSH) mutant protein which binds to Sertoli cells was used as a testis-specific carrier in the study. The occludin peptide linked to the mutant FSH induced transient and reversible disruption of the blood–testis barrier without compromising the TJ-barrier integrity in epithelia of kidney, liver, and small intestine [90]. This study indicates that targeting of peptides interacting with TJ proteins by receptor ligands may confer organ selectivity to barrier opening.

Disturbance of TJ function by directly targeting TJ proteins cannot only influence the flux of solutes, but also cellular transmigration. A cyclic occludin peptide antagonist, peptide B, containing a conserved occludin cell adhesion recognition sequence consistently and significantly increased neutrophil leukocyte migration across human umbilical vein endothelial cell layers in a time- and dose-dependent manner [108]. The increased chemotaxis correlated with disorganization of occludin, but not VE-cadherin in endothelial cells [108].

Another approach to modify permeability is the enzymatic cleavage of TJ proteins. Der p1, a major allergen from the house dust mite *Dermatophagoides pteronyssinus* and a cysteine proteinase, causes cell detachment in cultured kidney and tracheal epithelial cell layers and increases the permeability of isolated sheets of bronchial mucosa [92,150]. The time-dependent reversible disruption of TJ morphology

and ZO-1 redistribution from junctions to the cytoplasm were associated with cleavage of occludin [92]. The substrate of Der p1 is the first extracellular loop of human occludin, and potential cleavage sites exist in both extracellular loops of claudin-1 [92].

#### 4.2. Claudins

Sequence similarities between claudin-1 and -2 and the cloned receptor for *Clostridium perfringens* enterotoxin (CPE) causing food poisoning led to the identification of the receptor for CPE as claudin-4 [153]. CPE can also bind to claudin-3, -6, -7, -8, and -14 [154], the majority of claudins found in the GI tract. The C-terminal fragment of CPE (C-CPE), the binding domain of the toxin, selectively removed claudin-4 from TJs and disintegrated TJ strands in MDCK epithelial cells [95]. Parallely with the morphological changes the TJ barrier was downregulated in a time- and dose-dependent manner [95]. A peptide sequence containing the C-terminal 16 amino acids of C-CPE (Table 2) was found to be responsible for the interaction with the second extracellular domain of claudin-4 and the disruption of TJs [109,110]. Treatment of Caco-2 cells with C-CPE resulted in a decrease in TEER [110]. C-CPE enhanced the absorption of 4 kDa and 10 kDa dextran (but not that of 20 and 40 kDa) in rat jejunum [42,109,117]. C-CPE at 0.1 mg/ml concentration was as effective absorption enhancer as sodium caprate used in 40 mg/ml concentration, while no mucosal toxicity was observed [43,84,117]. The absorption enhancing effect of C-CPE was observed in the jejunum, but not in colon of rats, in contrast to sodium caprate, which was effective in both regions [117].

These findings indicate that claudin-4 can be a target of TJ modulators, like C-CPE. Other antagonists of claudin-4, including antibodies, antibody fragments, and small interfering nucleic acids may also have the potential to open epithelial TJs and to enhance paracellular transport (Table 1) [94].

#### 4.3. Zonulin

Zonula occludens toxin (Zot) of *V. cholerae* (Table 2) reversibly increases intestinal permeability by interacting with a surface receptor, activating PKC- $\alpha$  leading to disassembly of TJs [99]. Human zonulin was identified as the endogenous mammalian analogue of Zot, sharing a conserved N-terminal sequence corresponding to the putative receptor binding site [113]. A 45 kDa glycoprotein binding Zot and zonulin was demonstrated in brain [155], in the epithelium of the nasal region and the small intestine [101]. This correlates well with the *in vivo* tissue specificity of Zot, which is active only on the mucosal side of endothelial cells and epithelial cells in the nasal region, the jejunum and ileum, but not in the colon or kidney (Table 3) [156]. Zot and zonulin also bind  $\beta$ -tubulin, and this interaction can contribute to their TJ regulating action [157].

The TJ modulator and AE effects of Zot and active fragments derived from it,  $\Delta$ G and AT1002 peptides, were demonstrated on several models (Table 3), including nasal and intestinal epithelium and cultured brain endothelial cells. Zot reversibly enhanced the intestinal permeability to insulin and immunoglobulins in rabbits [100]. Zot also induced a rapid and reversible decrease in TEER of brain endothelial monolayers and an increase in paracellular permeability for markers sucrose and inulin, and P-glycoprotein (Pgp) efflux pump ligand drugs doxorubicin and paclitaxel [158]. Zot also acts as an adjuvant for mucosal antigen delivery and induced protective immune responses to ovalbumin and tetanus toxoid through the intranasal and rectal routes [139]. The Zot fragment  $\Delta$ G enhanced the oral bioavailability of hydrophobic drugs interacting with Pgp, such as cyclosporin A, ritonavir, saquinavir and acyclovir [115]. AT1002, a 6-mer synthetic peptide fragment of Zot, enhances the *in vivo* intestinal absorption of cyclosporin A in rats [116], and the nasal absorption of large hydrophilic markers 4 kDa polyethylene glycol (PEG) and inulin [144]. Beside the intestinal and nasal routes,

AT1002 can potentially facilitate the pulmonary delivery of therapeutic agents (Table 3) [103].

Although the Zot and zonulin receptor and its signalling cascade leading to TJ opening have not been characterized fully yet, active peptide fragments of Zot seem to be promising agents to safely and effectively improve drug and vaccine delivery across mucosal barriers and the BBB.

#### 4.4. E-cadherin

Peptides, derived from the E-cadherin EC-1 domain containing HAV amino acid sequence can inhibit E-cadherin mediated cell-cell adhesion and modulate the intercellular junctions of cell monolayers [45,97]. The calcium-mediated cell adhesion of cultured brain endothelial cells was prevented by HAV peptide [45]. Beside HAV peptides, corresponding to the groove region of EC-1 domain, ADT sequences containing peptides of the bulge region of E-cadherin were identified, and they were also able to increase paracellular permeability in MDCK cells [97]. HAV and ADT hexapeptides (Table 2), interfering with the homophilic binding of E-cadherin, could time and dose-dependently reduce TEER and increase the paracellular transport of mannitol across the epithelial cell layers [97].

#### 4.5. Other targets

In a recent paper four groups of lipids, sphingosines, alkylglucosides, oxidized lipids and ether lipids, have been identified as non-toxic and reversible TJ modulators [85]. Treatment of bronchial/tracheal epithelial tissues with individual lipids, among them 1-1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine (PGPC) resulted in a rapid reduction of TEER, up to 95%, which was restored within 1 h. Lipid treatment could also increase the permeability of the barrier tissue for fluorescein isothiocyanate (FITC)-labelled 3 kDa dextran and for the 4 kDa human peptide YY3–36 [85]. No changes in TJ structural morphology were found by immunofluorescence microscopy. Although the exact mode of action of these lipid TJ modulators are not disclosed, cell membrane lipid composition and/or lipid rafts cannot be excluded as their targets.

A novel tight junction modulator peptide, PN159 (Table 2), has been described and tested by the same group [83,85,145]. PN159 shows a dose-dependent reduction of TEER with a rapid onset, quick recovery of the barrier functions on removal and good cell viability and low cytotoxicity. Parallely with the changes in TEER, PN159 enhanced the permeability for galantamine, calcitonin, human parathyroid hormone 1–34 and peptide YY3–36 on an *in vitro* tissue model (Table 3) [85,145]. In a rabbit model PN159 enhanced the systemic bioavailability of human peptide YY<sub>3–36</sub> via intranasal administration [145]. The specific molecular target of PN159 has not been published, but the extracellular domain of an integral membrane TJ protein cannot be excluded, since the peptide was identified by phage display on EGTA-treated human bronchial epithelial cells [83]. PN159 can be a safe and potent TJ modulator to enhance drug delivery by nasal and gastrointestinal routes of administration [85]. Several peptides from the PN series (Table 2) may have a TJ modulator effect similar to PN159 (Table 3).

On the analogy of claudin-4 modulators, antagonists of JAMs, peptides, antibodies, and small interfering nucleic acids, may also regulate paracellular permeability of TJs [94].

### 5. TJ modulating effects of absorption enhancers/paracellular permeability enhancers

The controlled and reversible opening of tight junctions in the biological barriers to enhance drug delivery by safe and effective AEs or paracellular permeability enhancers (PPEs) is a long time goal in pharmaceutical and biomedical research (Table 4). However, the



**Table 3**  
Tight junction modulators used in epithelial barriers of peripheral organs

Modulator substance	Organ/tissue/cell culture	Drug/tracer	Reference
<i>Gastrointestinal tract epithelium</i>			
<i>Duodenal</i>			
ΔG (Zot fragment; 12 kDa)	<i>In vivo</i> , rat intraduodenal cannula	Cyclosporin A, saquinavir; ritonavir, acyclovir	[115]
AT-1002	<i>In vivo</i> , rat intraduodenal cannula	Cyclosporin A, protease inhibitors	[116]
<i>Jejunal</i>			
Zot (45 kDa)	<i>Ex vivo</i> , rabbit, Ussing	Insulin, immunoglobulins, PEG 4000	[100]
Zonulin (47 kDa)	<i>Ex vivo</i> , Rhesus monkey, Ussing	[Decreased tissue resistance]	[113]
C-CPE	<i>In situ</i> , rat, loop assay	FITC-dextran 4 kDa, 10 kDa	[109,117]
Sodium caprate	<i>In situ</i> , rat, loop assay	FITC-dextran 4 kDa	[117]
Dimethyl-β-cyclodextrin	<i>In situ</i> , rat, loop assay	Insulin	[118]
NO donors (NOC5, NOC12)	<i>Ex vivo</i> , rat, Ussing chamber	5(6)-carboxyfluorescein	[119]
NO donors (SNAP, NOC5 and 12)	<i>Ex vivo</i> , rat, Ussing chamber	Insulin, eel calcitonin	[120]
NO donors (SNAP, NOC5 and 12)	<i>In situ</i> , rat, loop assay	5(6)-carboxyfluorescein	[121]
<i>Ileal</i>			
Zot (45 kDa)	<i>Ex vivo</i> , rabbit, Ussing	[Decreased tissue resistance]	[99]
Zot (45 kDa)	<i>Ex vivo</i> , rabbit, Ussing	Insulin, immunoglobulins, PEG 4000	[100]
Zonulin (47 kDa)	<i>Ex vivo</i> , Rhesus monkey, Ussing	[Decreased tissue resistance]	[113]
Sodium caprate	<i>In vivo</i> , rat	Polysucrose	[122]
NO donors (SNAP, NOC5 and 12)	<i>Ex vivo</i> , rat, Ussing chamber	Insulin, eel calcitonin	[120]
<i>Colonic</i>			
Zonulin (47 kDa)	<i>Ex vivo</i> , Rhesus monkey, Ussing	[Decreased tissue resistance]	[113]
Zot (45 kDa)	<i>In vitro</i> , Caco-2 monolayers	Mannitol, sucrose, inulin, PEG 4000	[123,124]
Zot (45 kDa)	<i>In vitro</i> , Caco-2 monolayers	Paclitaxel, doxorubicin, cyclosporin A, acyclovir, enaminones	[123,124]
Lipopeptide OP <sub>90–103</sub>	<i>In vitro</i> , Caco-2 monolayers	TEER↓, mannitol	[88]
C-CPE	<i>In vitro</i> , Caco-2 monolayers	TEER↓, FITC-dextran 4 kDa	[109]
EDTA	<i>In vitro</i> , Caco-2 monolayers	carboxyfluorescein, PEG 4000, furosemid, FITC-dextran	[125,126]
Oleic acid	<i>In vitro</i> , Caco-2 monolayers	TEER↓ mannitol	[127,128]
Sodium caprate	<i>In situ</i> , rat, loop assay	FITC-dextran 4 kDa	[117]
Sodium caprate	<i>In situ</i> , rat, loop assay	5(6)-carboxyfluorescein	[121]
Sodium caprate	<i>In vitro</i> , Caco-2 monolayers	Mannitol	[129,130]
Long-chain acylkarnitine	<i>Ex vivo</i> , rat	TEER↓	[131]
Long-chain acylkarnitine	<i>In vitro</i> , Caco-2 monolayers	TEER↓	[132]
Sodium dodecyl sulfate	<i>In vitro</i> , Caco-2 monolayers	TEER↓ mannitol	[133,134]
Polysorbate 20, Solulans	<i>In vitro</i> , Caco-2 monolayers	TEER↓ metformin	[135]
Nonylphenol ethoxylates	<i>In vitro</i> , Caco-2 monolayers	Mannitol, daunomycin	[136]
Bile salts	<i>In vitro</i> , Caco-2 monolayers	TEER↓ mannitol, PEG	[133]
Chitosans	<i>In vitro</i> , Caco-2 monolayers	TEER↓ inulin	[137,138]
NO donors (NOC5, NOC12)	<i>Ex vivo</i> , rat, Ussing chamber	5(6)-carboxyfluorescein	[119]
NO donors (SNAP, NOC5 and 12)	<i>Ex vivo</i> , rat, Ussing chamber	Insulin, eel calcitonin	[120]
NO donors (SNAP, NOC5 and 12)	<i>In situ</i> , rat, loop assay	5(6)-carboxyfluorescein	[121]
<i>Rectal</i>			
His-Zot	<i>In vivo</i> , mouse	Tetanus toxoid	[139]
<i>E. coli</i> heat labile enterotoxin	<i>In vivo</i> , mouse	Tetanus toxoid	[139]
Sodium caprate	<i>In vivo</i> , human	Ampicillin	[140]
Cyclodextrins	<i>In vivo</i> , rabbit	Insulin	[141]
NO donors (SNAP, NOR1 and 4)	<i>In vivo</i> , rabbit	Insulin	[142]
<i>Airway epithelium</i>			
<i>Nasal</i>			
His-Zot	<i>In vivo</i> , mouse	Ovalbumin, tetanus toxoid	[139]
MBP-Zot	<i>In vivo</i> , mouse	Ovalbumin, tetanus toxoid	[139]
Zot	<i>In vivo</i> , mouse	Gliadin	[143]
AT-1002	<i>In vivo</i> , rat	PEG 4000, inulin	[144]
<i>E. coli</i> heat labile enterotoxin	<i>In vivo</i> , mouse	Ovalbumin, tetanus toxoid	[139]
PN 159	<i>In vivo</i> , rabbit	Peptide YY <sub>3–36</sub>	[104,105,145]
PN 27, 58, 73, 202, 183, 228, 556	<i>In vivo</i> , rabbit	Peptide YY <sub>3–36</sub>	[105]
EGTA	<i>In vivo</i> , human	TEER↓	[146]
Bile salts	<i>In vitro</i> , human NEC	fexofenadine	[147]
Cyclodextrins	<i>In vivo</i> , rabbit	Insulin	[148]
Dimethyl-β-cyclodextrin	<i>In vivo</i> , rat	TEER↓ mannitol, enoxaparin	[149]
NO donor SNAP	<i>In vivo</i> , rabbit	rh Granulocyte colony-stimulating factor	[148]
<i>Pulmonary</i>			
PN 159	<i>In vitro</i> , human REC	Calcitonin, parathormone, galantamine, MC-4RA	[104,105]
PN 159, PN393, PN407,	<i>In vitro</i> , human REC	FITC-dextran (3 kDa), TEER↓	[104]
PN679, PN745, PN434, PN408	<i>In vitro</i> , human REC	FITC-dextran (3 kDa)	[105]
AT-1002	<i>In vitro</i> , human REC	FITC-dextran (4 kDa), TEER↓	[103]
AT-1002	<i>In vivo</i> , rat (instillation to lung)	Calcitonin	[103]
FDFWTP 7-mer peptide	<i>In vitro</i> , human REC	TEER↓	[107]
Lipopeptide OP <sub>90–103</sub>	<i>In vitro</i> , human REC	TEER↓, FITC-dextran (70 kDa), adenovirus vector	[89]
Der p1 cysteine proteinase	<i>In vitro</i> , canine TEC	Albumin	[92,150]
PGPC	<i>In vitro</i> , human REC	TEER↓ FITC-dextran (3 kDa), peptide YY <sub>3–36</sub>	[85]
EDTA/EGTA/BAPTA	<i>In vitro</i> , human REC	TEER↓ gene transfer	[146]
EGTA	<i>In vivo</i> , rabbit trachea	TEER↓	[146]
EGTA	<i>In vitro</i> , human REC	TEER↓ mannitol, dextran, adenoviral transfer	[151]

**Table 3** (continued)

Modulator substance	Organ/tissue/cell culture	Drug/tracer	Reference
<b>Airway epithelium</b>			
<b>Pulmonary</b>			
Oleic acid	<i>In vitro</i> , human REC	mannitol	[152]
Sodium caprate	<i>In vitro</i> , human REC	TEER↓, mannitol, dextran, adenoviral transfer	[151]
<b>Blood–testis barrier</b>			
ΔFSH-fused occludin peptide	<i>In vivo</i> , rat	FITC-inulin	[90]

**Abbreviations:** BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; BEC, bronchial epithelial cells; C-CPE, C-terminal fragment of *Clostridium perfringens* enterotoxin; EDTA; ethylenediamine-N,N,N',N'-tetraacetic acid; EGTA, ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid; FITC, fluorescein isothiocyanate; FSH, follicle stimulating hormone; His-Zot, zonula occludens toxin tagged at the N-terminus with a hexahistidine tail; MBP-Zot, zonula occludens toxin fused with the maltose-binding protein; MC-4RA, melanocortin-4 receptor agonist; NEC, nasal epithelial cells; NOC5, [3-(2-hydroxy-1-(methylethyl)-2-nitrosohydrazino)-1-propanamine]; NO, nitric oxide; NOC12, [N-ethyl-2-(1-ethyl-hydroxy-2-nitrosohydrazino)-ethanamine]; NOR1, (+/–)-(E)-4-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide; NOR4, (+/–)-N-[(E)-4-Ethyl-2-[(Z)-hydroxyimino]-5-nitro-3-hexene-1-yl]-3-pyridine carboxamide; PEG 4000, polyethylene glycol 4 kDa; REC, respiratory epithelial cells; rh, recombinant human; SNAP, S-nitroso-N-acetyl-DL-penicillamine; TEC, tracheal epithelial cells; TEER↓, decrease in transepithelial electrical resistance; Zot, zonula occludens toxin (45 kDa).

knowledge about the molecular composition and regulation of TJs was scarce in the past. The first integral membrane protein of the TJ complex, occludin was discovered in 1993 [25] and in the last 15 years a great progress has been achieved with the discovery of new TJ, AJ and adaptor proteins and protein families, signalling molecules and pathways [3]. Because of this fact AEs and PPEs developed before the discovery of the molecular composition and function of TJs have a rather unspecific mode of action (Table 4). Most importantly, the difference between the effective and toxic doses of these AEs is small [7]. Despite these drawbacks some of the AEs, e.g. sodium caprate (C10), are effective and clinically used in oral formulations.

Some of the AEs enhancing transdermal drug delivery, like surfactants and fatty acids, are able to increase buccal permeability. However, the mode of action of AEs is dissimilar in the skin and buccal mucosa due to the different morphological and molecular structure of the two barriers [177]. Therefore, AEs might be effective in one barrier,

but not in the other, and although they are able to act on several barriers, their local action can be different.

### 5.1. Chelators

Calcium chelators, like ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA), ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) increase paracellular permeability through disruption of AJs and TJs via activation of PKC [159]. EDTA enhances the permeability of Caco-2 monolayers for polyethylene glycol 4000 [125], furosemide, carboxyfluorescein [126], and FITC-dextran without serious cytotoxicity [178]. EDTA, EGTA and BAPTA in a hypotonic buffer caused a rapid, reversible drop in TEER and facilitated gene transfer with retrovirus or adenovirus from the apical side on cultured human differentiated airway epithelial cells by opening the intercellular TJs [146]. Chelators applied to rabbit tracheal epithelia or human nasal

**Table 4**

Absorption enhancers, miscellaneous tight junction modulators and non-specific targets

Modulator substance	Possible target/mechanism	Reference
<b>Chelators</b>		
EGTA, EDTA, BAPTA	Ca <sub>i</sub> , PKC, AJ and TJ disassembly	[159]
<b>Fatty acids, modified fatty acids, phosphate esters, phospholipids</b>		
Oleic acid	Lipid rafts, PKC, gap junction	[160,161]
Sodium caprate	PLC, PI3, redistribution of TJ proteins	[129,130]
Palmitoyl carnitine	PKC, gap junction	[162]
Lysophosphatidic acid	PKC, MAPK, gap junction, redistribution of TJ proteins	[163,164]
<b>Surfactants</b>		
Sodium dodecyl sulfate	Actin reorganization, Ca <sub>i</sub> , AP-1 and NF-κB	[134,165]
Bile salts: sodium cholate, dehydrocholate, taurocholate	Ca <sub>i</sub> , F-actin reorganization	[147,166]
<b>Cationic polymers, polycations</b>		
Protamine	Negative surface charge↓, actin reorganization; occludin, claudin-1↓	[167,168]
Poly-L-Lys, poly-L-Arg	PKC, phospholipase D	[169]
Chitosan	PKC-α, redistribution of actin, occludin, ZO-1	[137,138,170,171]
<b>Cyclodextrins</b>		
Dimethyl-β-CD	Cholesterol depletion, lipid rafts, TJ disruption	[172,173]
<b>Nitric oxide donors, bradykinin</b>		
Cereport, DEA-NONOate, PAPA-NONOate	Ca <sub>i</sub> , cyclic GMP↑, K <sub>Ca</sub> channel activation, TJ opening	[174]
<b>Hyperosmotic solutions</b>		
Mannitol	Src kinase, β-catenin phosphorylation, redistribution of TJ proteins	[175]
<b>High frequency focused ultrasound</b>		
MRI-guided high frequency focused ultrasound/Optison	Vasoconstriction, transcytosis, TJ disruption	[176]

**Abbreviations:** AJ, adherens junction; AP-1, activator protein 1 transcription factor; BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; Ca<sub>i</sub>, removal of extracellular calcium ions; Ca<sub>i</sub>↑, increase in intracellular calcium ion concentration; CD, cyclodextrin; cyclic GMP, cyclic guanosine monophosphate; DEA-NONOate, diethylammonium (Z)-1-(N,N-diethylamino)diazene-1-ium 1,2-diolate; EDTA; ethylenediamine-N,N,N',N'-tetraacetic acid; EGTA, ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid; K<sub>Ca</sub>, calcium-activated potassium channel; MAPK, mitogen activated protein kinase; MRI, magnetic resonance imaging; NF-κB, nuclear factor-κB transcription factor; PAPA-NONOate, (Z)-1-[N-(2-aminopropyl)-N-(2-ammonio propyl)amino]diazene-1-ium 1,2-diolate; PI3, phosphatidylinositol 3,4,5-triphosphate; PKC, protein kinase C; PLC, phospholipase C; TJ, tight junction; ZO-1, zonula occludens protein 1.

epithelia *in vivo* decreased the transepithelial voltage and amiloride sensitivity, suggesting that epithelial junctions opened. Importantly, this novel formulation enhanced both retroviral- and adenoviral-mediated gene transfer to rabbit tracheal epithelia *in vivo* [146]. Chelation of calcium to open epithelial apical junctions could allow the targeting of airway cells with progenitor capacity that are located below the surface layer to attain persistent gene transfer [146]. This study indicates the potential clinical usefulness of chelators as TJ modulators.

### 5.2. Fatty acids, modified fatty acids and phosphate esters

Oleic acid is one of the most popular AEs, used in transdermal drug delivery vehicles, patches, and microemulsions [179,180]. It is known to modulate the structure of membranes. Recent data indicate, that it does not perturb significantly the structure of lipid bilayers, and its effects can be restricted to more condensed membrane structures, such as the gel phase, more characteristic to cholesterol-containing ordered domains, like lipid rafts [160]. Oleic acid decreased TEER [127,128] and increased mannitol flux in Caco-2 cells, parallelly with disintegration of cell–cell contacts [128] (Tables 3, 4). Oleic acid also enhanced the permeability of mannitol across cultured alveolar epithelial cells in a calcium-dependent way [152]. Beside its effect on epithelial barrier, reversible opening of the BBB was induced by oleic acid or oleic acid emulsion injected to the carotid artery in animal studies [181,182]. The data listed above suggest that beside its effect on membrane fluidity, oleic acid can increase paracellular permeability of dermal, gastrointestinal, alveolar and blood–brain barriers [127,128,152,179–182]. The exact target of oleic acid is not known, but membrane microdomains or lipid rafts could be among them [160].

Sodium caprate, a medium chain fatty acid (C10), and a constituent of milk fat, is an absorption enhancer used in clinical therapy. It enhanced the permeability of mannitol in Caco-2 monolayers by redistribution of the cytoskeleton, TJ proteins ZO-1 and occludin, and opening of TJs through phospholipase C-dependent inositol triphosphate/diacylglycerol pathways [129,130,183] (Table 4). Sodium caprate also increased TJ permeability and the transport of polysucrose in rat ileum [122], and ampicillin bioavailability after rectal administration in humans [140]. It should be noted, that reversible mucosal damage could be observed after sodium caprate exposure [140]. On another type of barrier, sodium caprate-induced TEER drop, increased mannitol and dextran permeability, adenoviral *lacZ* gene transfer was more effective in airway epithelial cells than the EGTA-induced changes [151]. A redistribution of claudin-1, found following C10 but not EGTA treatment, is hypothesized as a possible mechanism of gene-transfer enhancement by C10 [151].

Palmitoyl carnitine, a long-chain (C16) fatty acid ester, greatly enhances the absorption of hydrophilic drugs across intestinal mucosa [131], and across Caco-2 epithelial cells accompanied by a rapid and reversible drop in TEER and opening of TJs [132] (Table 3). However, transport enhancement was accompanied by an increase in apical membrane permeability and a reduction in cell viability [184] indicating insufficient separation between efficacy and toxicity.

### 5.3. Surfactants

Surfactants are solubilizing excipients widely used in oral, injectable and nasal formulations [185,186]. Anionic, non-ionic synthetic surfactants and bile salts have been extensively studied to enhance transepithelial permeability for different marker molecules, peptides and drugs (Table 3), but their effect on TJs is less well characterized (Table 4).

Sodium dodecyl sulfate (SDS), an anionic surfactant had an immediate effect on paracellular permeability of Caco-2 cells, the TEER dropped, intracellular calcium levels increased, and TJs separated [133,134]. While short-term incubation of cells with SDS caused reversible enhancement of mannitol permeability, longer exposure resulted in irreversible changes and apical membrane injury and

cellular toxicity [133,134]. The toxicity of surfactants polyoxyethylene 9 lauryl ether and sodium glycocholate given at high concentration (1%) was demonstrated after administration to rat lungs, where they induced acute inflammation [187].

Non-ionic surfactants polysorbate 20, oxyethylene ether Solulan C24 and C16 showed a concentration dependent paracellular increase in metformin transport, parallelly with TEER decrease, however, increased permeability correlated with decreased cell viability of Caco-2 cells [135]. Nonylphenol ethoxylates can also rapidly and reversibly open paracellular TJs and enhance the transport of mannitol and daunomycin, but these non-ionic surfactants also cause irreversible changes in long-term treatments [136].

Bile salts, sodium cholate, sodium taurocholate and sodium taurodeoxycholate, which are ionic surfactants, show also a concentration-dependent effect on epithelial permeability and morphology in Caco-2 cells and human nasal epithelial cells, respectively [133,147]. A dose dependent reduction of cell viability was also observed at higher than critical micelle concentration, but bile salts exerted less toxicity, than non-ionic surfactants Tween 80 and Poloxamer F68 [147].

### 5.4. Cationic polymers

Cationic polymers, like poly-L-lysines, polyethyleneimine and chitosan, are able to induce reversible opening of TJs in epithelial cell models [137,188,189]. Chitosan and its derivatives are non-toxic biocompatible polymeric AEs obtained from chitin. Because of their mucoadhesive as well as AE properties these cationic polysaccharides have been extensively studied as excipients for drug delivery across dermal, gastrointestinal, nasal, and pulmonary epithelial barriers (Table 3) [186,190–192]. The chitosan-induced reversible increase in TJ permeability involves the redistribution of occludin, ZO-1 and the actin-cytoskeleton [137,170,171]. The process is mediated by activation of PKC- $\alpha$  in Caco-2 cells [138,193]. Because of their low toxicity, good water solubility, mucoadhesive properties in addition to their TJ modulator effect, chitosan derivatives are promising candidates to enhance drug delivery in clinical setting.

### 5.5. Cyclodextrins

Cyclodextrins (CDs) are natural cyclic oligosaccharides derived from starch and used as excipients in marketed pharmaceutical products [194]. CDs in pharmaceutical products are mainly used as complexing agents. The potential of CDs to be used as penetration enhancers for drugs has been investigated on several models [118,141,148,149,170,194,195] (Table 3). Dimethyl- $\beta$ -CD is a potent enhancer of nasal and intestinal absorption of heparins and insulin *in vivo* and *in vitro* [118,141,148,149,170,195]. The dimethyl- $\beta$ -CD-induced increase in the permeability for mannitol and enoxaparin was accompanied by reduction of TEER and changes in ZO-1 distribution in epithelial cells, indicating opening of TJs [149]. Dimethyl- $\beta$ -CD was found to be more effective to enhance bioavailability and absorption of heparin and insulin than all other CDs tested, hydroxypropyl- $\beta$ -CD,  $\alpha$ -,  $\beta$ - $\gamma$ -CD [141,148,149,195]. This effect seems to correlate with the cholesterol depleting efficacy of CDs [172,173,196] (Table 4). Beside other well characterized effects of CDs on drug complexation and unstirred water layer [194], cholesterol depletion from epithelial cell membrane, especially from lipid rafts, and subsequent loss of TJ integrity, displacement of TJ proteins can explain the AE effect of dimethyl- $\beta$ -CD [81,82]. However, the cholesterol depleting effect of CDs also correlate with their cytotoxicity on epithelial and BBB *in vitro* models [172,196,197].

### 5.6. Nitric oxide donors

NO is a regulator of epithelial TJs, and excessive levels of NO generated by activated inducible NO synthase result in epithelial

barrier dysfunction in the lung, liver and gut [198]. Decreased expression and dislocation of TJ proteins occludin, ZO-1, -2 and -3 are accompanied by enhanced transport of FITC-dextran across the ileal mucosal membrane were observed [199].

NO donor molecules, S-nitroso-N-acetyl-DL-penicillamine (SNAP), (2-hydroxy-1-methylethyl-2-nitrosohydrazino)-N-methyl-1-propanamine (NOC7), (2-hydroxy-1-(1-methylethyl)-2-nitrosohydrazino)-1-propanamine (NOC5), and N-ethyl-2-(1-ethyl-2-hydroxy-2-nitrosohydrazino)-ethanamine (NOC12) have been tested as potential AEs in epithelial barriers of the gastrointestinal tract and in the nasal mucosa [119–121,142,200–202] (Table 3). SNAP enhanced the intestinal absorption of insulin, and calcitonin in rabbits and rats, and the nasal absorption of human granulocyte colony-stimulating factor in rabbits [120,142,201]. When compared with other AEs, like sodium glycocholate and sodium caprate, SNAP was the most effective AE in the small intestine showing excellent effectiveness at low concentra-

tion [121]. However, the exact mechanism of action is not known, and the hypothesized effect on TJs was not examined yet.

## 6. Modulation of TJs at the blood–brain barrier for drug targeting

### 6.1. Modulators acting on brain endothelial TJs

There are no data yet on the effect of the newly discovered epithelial TJ modulators on the BBB, except for Zot and its fragments (Table 5). Integral membrane TJ proteins occludin, JAMs, claudin-5, AJ proteins VE-cadherin and the BBB-specific cadherin-10 [233], or AHNK colocalizing in TJs at the BBB [234], could be all potential targets able to modulate paracellular permeability in brain endothelial cells. C-CPE is an unlikely TJ modulator for the CNS, because its target, claudin-4 is absent at the BBB. On the other hand, lipid modulators, like PGPC may potentially open the BBB, however, no data exist on their effects on BBB models.

**Table 5**

Modulators used for the opening of the tight junctions at the blood–brain barrier

Modulator substance	Organ/tissue/cell culture	Drug/tracer	Reference
<i>Modulators acting on tight junctions</i>			
Zonula occludens toxin (45 kDa)	<i>In vitro</i> , bovine BMVEC	Sucrose, inulin	[158]
Zonula occludens toxin (45 kDa)	<i>In vitro</i> , bovine BMVEC	Doxorubicin, paclitaxel	[158]
ΔG; 12 kDa (Zot active fragment)	Intracarotid injection, rat	Methotrexate, paclitaxel	[203]
18β-glycyrrhetic acid, oleamide	<i>In vitro</i> , porcine BMVEC	Mannitol, inulin	[204]
Cereport (lobradimil)	<i>In vitro</i> , human BMVEC	Inulin	[69]
Cereport (lobradimil)	Glioma and PD model, rat	Loperamide, cyclosporin A	[205–207]
Cereport (lobradimil)	Glioma model, rat	Carboplatin, paclitaxel, doxorubicin	[207,208]
Cereport (lobradimil)	Clinical trials, human	Carboplatin	[209]
Histamine	<i>In vitro</i> , bovine BMVEC	Sucrose, inulin, EBA	[66]
Histamine	Glioma model, rat	GABA, EBA	[210]
<i>Absorption enhancers and new therapeutical approaches</i>			
<i>Chelators</i>			
EGTA	<i>In vitro</i> , bovine BMVEC	Sucrose	[212]
BAPTA	<i>In vitro</i> , porcine BMVEC	Sucrose	[163]
<i>Fatty acids, modified fatty acids, phospholipids</i>			
Oleic acid	Intracarotid injection, rat	γ-AIB, EBA	[181]
Linoleic acid and oleic acid emulsion	Intracarotid injection, cat	Gadolinium	[182]
Sodium caprate	Intracarotid injection, rat	Mannitol	[213]
Lysophosphatidic acid	<i>In vitro</i> , porcine BMVEC	TEER↓	[163]
<i>Surfactants</i>			
Sodium dodecyl sulfate	Intracarotid injection, rat	γ-AIB, EBA	[214]
Sodium dehydrocholate	Intracarotid injection, rat	Fluorescein, EBA	[215,216]
Sodium dehydrocholate, TCDC	<i>In situ</i> brain perfusion, rat	Mannitol [217]	[217]
Sodium dihydroxy-oxo-cholanate	Subcutaneous injection, rat [218]	Quinine, morphine, pentobarbital	[218]
<i>Cationic polymers</i>			
Protamine	<i>In situ</i> brain perfusion, rat	HRP	[167]
Protamine, poly-L-Lys, poly-L-Arg	Intracarotid injection, rat	Albumin	[219]
Chitosan	Intravenous injection, mouse	Nobiletin	[220]
<i>Cyclodextrins</i>			
α-CD, β-CD, γ-CD	<i>In vitro</i> , bovine BMVEC	Sucrose	[172]
Hydroxypropyl-γ-CD, γ-CD	<i>In vitro</i> , bovine BMVEC	Doxorubicin	[173]
Rame-β-CD, chrisme-β-CD	<i>In vitro</i> , bovine BMVEC	Doxorubicin	[221]
<i>Nitric oxide donors</i>			
SNAP	<i>In situ</i> brain perfusion, rat	Sucrose	[222]
DEA-NONOate, PAPA-NONOate	Intracarotid, RG2 tumour, rat	γ-AIB	[174]
<i>Hyperosmotic solutions</i>			
Arabinose	<i>In vitro</i> , bovine BMVEC	Sucrose	[223]
Arabinose	Intracarotid injection, rat	TJ opening	[224]
Mannitol	<i>In vitro</i> , bovine BMVEC	Sucrose, inulin	[225]
Mannitol	Intraarterial injection, human	Anticancer drugs	[226]
<i>Short-chain alkylglycerols</i>			
1-O-pentylglycerol	Intracarotid injection, rat and mice	Fluorescein, albumin	[227,228]
1-O-pentylglycerol	Intracarotid, C6 or RG2 tumour, rat	Cisplatin, methotrexate, antibiotics	[228]
<i>High frequency focused ultrasound</i>			
High frequency focused ultrasound	Rabbit	MRI contrast agents Magnevist, Mion-47	[229]
High frequency focused ultrasound	Mouse	Antibodies	[230,231]
High frequency focused ultrasound	Rat	Doxorubicin	[232]

**Abbreviations:** BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; BMVEC, brain microvascular endothelial cell monolayer; CD, cyclodextrin; DEA-NONOate, diethylammonium (Z)-1-(N,N-diethylamino)diazene-1-ium 1,2-diolate; ΔG, zonula occludens toxin active fragment (12 kDa); EBA, Evans blue albumin; EGTA, ethylene glycol-bis (beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid; GABA, γ-aminobutyric acid; γ-AIB, γ-aminoisobutyric acid; HRP, horseradish peroxidase; MRI, magnetic resonance imaging; PAPA-NONOate, (Z)-1-[N-(2-aminopropyl)-N-(2-ammonio propyl)amino]diazene-1-ium 1,2-diolate; PD, Parkinson's disease; SNAP, S-nitroso-N-acetyl-DL-penicillamine; TCDC, taurochenodeoxycholate; TEER, transendothelial electric resistance; TJ, tight junction; Zot, zonula occludens toxin.



Gap junction proteins connexin-40 and -43 are colocalized and coprecipitated with TJ proteins in brain endothelial cells [204]. Blockers of gap-junctions, 18 $\beta$ -glycyrrhetic acid and oleamide, impaired the barrier function of brain endothelial cells, decreased the TEER and increased the paracellular flux of mannitol and inulin [204]. Interestingly, no change in the expression and localization of occludin, claudin-5, JAMs or ZO-1 was detected after treatment with gap-junction inhibitors. These findings indicate, that gap-junctions can participate in the barrier function at the BBB and can be targets of TJ modulators.

There are several examples of receptor-mediated activation of signalling pathways leading to transient opening of TJs at the BBB (for review see [66]). A Zot-binding glycoprotein was purified from brain [155], and Zot is active as a TJ modulator at the brain endothelium. It induces a reversible, concentration-dependent TEER decrease, and an increase in the paracellular transport of the permeability markers sucrose and inulin, indicating TJ opening, without short-term toxicity in cultured brain endothelial cells [158]. Zot also enhanced the transport of chemotherapeutic agents doxorubicin and paclitaxel, ligands of Pgp [158]. The Zot active fragment  $\Delta$ G, given into the carotid artery of rats, increased several fold the brain penetration of sucrose, paclitaxel, and the hydrophilic methotrexate [203] (Table 5).

Vasoactive compounds, such as histamine, bradykinin, or leukotrienes are well-known mediators of brain oedema formation and increase BBB permeability [66,235,236]. Blood vessels in brain tumour tissue are more sensitive to the permeability enhancing effects of these mediators, than normal BBB [236]. Structural and functional changes found in the microvasculature of brain tumours are responsible for loss of BBB integrity (see for review [237]). Cultured glioblastoma cells and glioma-derived factors induced downregulation of the expression of occludin, claudin 1, and claudin 5, and an increase in paracellular permeability through brain endothelial cell monolayers [238].

Cereport (RMP-7, labradimil), a 9-mer synthetic peptide analogue of bradykinin increases intracellular calcium ion and cyclic GMP levels through bradykinin B2 receptors and induces a rapid and transient elevation in the paracellular transport of 5 kDa inulin in cultured brain endothelial cells [68,239]. Cereport opened brain endothelial TJs [240] and enhanced the CNS delivery of carboplatin, loperamide and cyclosporin-A [205–208] (Table 5). Cereport attached to liposomes facilitated Evans blue penetration to brain in rats [241]. Enhanced tumour uptake of chemotherapeutics and increased survival were found in Cereport-treated animals using rodent models of glioma and metastatic brain tumours [207,208]. After promising preclinical trials and Phase I studies, no benefit of Cereport was found in a Phase II study in childhood high-grade and brainstem gliomas [209]. Despite the failure of Cereport to potentiate chemotherapy of brain tumours, where high level of Pgp on the tumour cells restricts the efficacy of chemotherapy, Cereport can still be useful in enhancing drug delivery across BBB [205–208,241].

Histamine decreases TEER and increases the permeability for paracellular markers in BBB models *in vitro* [66] and *in vivo* [235]. Small dose of histamine injected to the carotid artery resulted in a selective extravasation of Evans blue and an increase in the transport of  $\gamma$ -aminoisobutyric acid in the brain tumour without changing BBB permeability in other regions [210,211]. The signalling pathway involves histamine H2 receptors, NO and cyclic GMP production [242,243]. A reversible and concentration-dependent reduction in TJ protein ZO-1 was observed in histamine-treated retinal endothelial cells, which show similar properties to BBB [244].

## 6.2. Permeability enhancers and new experimental approaches

Calcium and other divalent cations are needed for the integrity of brain endothelial TJs, similarly to epithelial cells. Removal of extracellular calcium ions by chelators BAPTA and EGTA (Table 5) results in a rapid opening of TJs in brain endothelial cells, a decrease in TEER, and an increase in sucrose flux [66]. However, chelators have not been tested for drug delivery to brain in cell culture or animal studies.

Fatty acids and modified fatty acids are effective absorption enhancers in epithelial barriers, and some of them have been also tested on the BBB. Reversible opening of the BBB to Evans blue and  $\gamma$ -aminoisobutyric acid was observed after intracarotid artery infusion of oleic acid in rats [181]. Since oleic acid is a PKC activator, an effect on signalling pathways regulating TJs should be considered. Emulsions made from oleic acid or linoleic acid injected to carotid artery of cats increased BBB permeability in a reversible manner detected by magnetic resonance imaging (MRI) [182]. Sodium caprate, a clinically used AE in the GI tract, when given as intracarotid infusion produced reversible, dose-related BBB opening for mannitol in rats [213] (Table 5). However, high doses were toxic and caused severe brain edema and cardio-respiratory failure [213].

The phospholipid LPA, found in animal sera used for cell cultures or released in high amount during subarachnoid haemorrhage, causes a rapid, reversible and dose-dependent decrease in TEER in brain endothelial cells [163,245]. The barrier weakening was accompanied by redistribution of occludin, claudin-5 and ZO-1 indicating that TJ proteins are directly affected by LPA-treatment [245].

The anionic surfactant SDS, widely used as solubilizer and stabilizer in pharmaceutical preparations, and a known AE, elicited an extensive, dose-dependent and reversible BBB opening in rats [214]. Intracarotid infusion of SDS caused Evans blue extravasation, and a significant increase in  $\gamma$ -aminoisobutyric acid transport to brain. Part of the effect is hypothesized to be mediated by TJ modulation [214]. The ionic surfactant bile salts, effective as AE in the GI tract also induce reversible BBB opening. Sodium dehydrocholate infusion to the carotid artery of rats disrupted the BBB for fluorescein and Evans blue in a concentration-dependent manner [215,216]. Sodium deoxycholate and taurochenodeoxycholate had the same effect when tested using *in situ* rat brain perfusion [217]. A modified bile salt, sodium dihydroxy-oxo-cholanate, enhanced BBB permeability for quinine, morphine and pentobarbital in rats, but its action on brain endothelial TJs is unspecified [218]. Because dehydrocholate and deoxycholate are strong detergents at high doses, and lytic action on endothelial cell membranes can be observed with the development of EEG changes and epileptiform activity [217,246,247], their possible clinical application is limited.

The negative surface charge of the luminal endothelial membrane of brain vessels contributes to the barrier phenotype and cationic molecules show better BBB permeability (for review see [66]). Intracarotid infusion of polycations protamine, poly-L-lysine and poly-L-arginine in rats resulted in permeability increase for albumin [219]. In a rat brain perfusion model protamine decreased the negative luminal surface charge in brain vessels and increased the extravasation of HRP by opening endothelial TJs [167]. The cationic polymer chitosan applied in microemulsion could improve nobiletin transport to brain in mice when given intravenously [220], although the mode of action or specific effects on TJs have not been investigated.

Native  $\alpha$ -CD- and  $\beta$ -CD-treatment causes a rapid increase in sucrose permeability of brain endothelial monolayers in parallel with their ability to extract phospholipids and cholesterol from the cell membranes [172]. Hydroxypropyl- $\gamma$ -CD and  $\gamma$ -CD at high concentration increased doxorubicin transport across brain endothelial monolayers parallelly with the loss of BBB integrity and decreased junctional staining of occludin [173]. Rame- $\beta$ -CD and chrisme- $\beta$ -CD were also able to enhance doxorubicin transport in the same *in vitro* BBB model (Table 5), and this effect correlated to the cholesterol extracting capacity of  $\beta$ -CDs, which may modulate TJs and the activity of efflux pumps in the brain endothelial cell membranes [221].

While basal NO production is necessary for the BBB integrity, excessive NO release either by inducible NO synthase or by NO donors leads to increased permeability in BBB models [66]. NO donor molecule SNAP enhanced the BBB sucrose permeability about 5-fold in all forebrain regions using an *in situ* rodent brain perfusion method [222]. Intracarotid infusion of diethylammonium (Z)-1-(N,N-diethylamino)diazen-1-ium 1,2-diolate (DEA-NONOate) and (Z)-1-[N-(2-aminopropyl)-N-(2-aminopropyl)amino]diazen-1-ium 1,2-diolate (PAPA-NONOate), short-

acting NO donors, in RG2 tumour bearing rats led to increased transport of  $\gamma$ -aminobutyric acid to the tumour tissue [174] (Table 5). The effect of DEA-NONOate and PAPA-NONOate on brain tumour vessel permeability was mediated by calcium-activated potassium channels [174] (Table 4).

Osmotic stress induced by mannitol or arabinose causes a rapid and reversible decrease in TEER, and increases permeability for paracellular markers sucrose and inulin through brain endothelial monolayers [66], and in animal models (for review see [248]). After intracarotid infusion of hyperosmotic arabinose dilatation of junctional clefts and changes in the localization and expression of TJ proteins occludin, claudin-5, JAM-1 and ZO-1 were observed in rats [224]. Src kinase mediated phosphorylation of AJ protein  $\beta$ -catenin may play a role in the mechanism of mannitol-induced TJ opening [175]. Ongoing multicenter clinical studies suggest that BBB disruption by intraarterial hyperosmotic mannitol can enhance the penetration of anticancer drugs and prolong survival in patients with malignant brain tumours [226] (Table 5). Despite successful application in some centers, the method has not been widely spread, because the procedure is difficult, invasive, and posttreatment neurotoxicity may develop.

Erdlenbruch et al. demonstrated, that the short chain alkylglycerol, 1-O-pentylglycerol, given to the carotid artery could rapidly and reversibly enhance the ipsilateral brain penetration of antineoplastic agents cisplatin and methotrexate and the antibiotics vancomycin and gentamycin in rats [249]. It also enhanced the delivery of erucylphosphocholine and methotrexate to rat C6 and RG2 implanted tumours [227,228,249,250]. 1-O-pentylglycerol induced the extravasation of fluorescein, albumin and methotrexate in the ipsilateral brain and to the tumour by enhancing paracellular permeability of TJs [228]. The effect of 1-O-pentylglycerol was lower than that of hyperosmotic mannitol, and much higher than the effect of bradykinin, but in contrast to mannitol it did not enhance BBB permeability in the contralateral brain hemisphere or the cerebellum and brain stem [227]. The effect and duration of alkylglycerol-induced BBB-opening was more rapid and localized to the site of injection, with less *in vivo* toxicity [227], offering a new and safer alternative of BBB disruption by hyperosmotic mannitol.

There are a growing number of papers describing experimental BBB opening by high intensity focused ultrasound (for review see [251]). Low frequency MRI-guided ultrasound bursts could induce local, reversible disruption of BBB in rabbits [229] and increase the brain delivery of dopamine D4 receptor antibody, and a humanized anti-human epidermal growth factor receptor 2 monoclonal antibody, Herceptin, in mice [230,231], and doxorubicin in rats [232] (Table 5). The method is not specific to TJs, the mechanisms of transport of molecules by sonication can involve transcytosis, endothelial fenestration and channel formation, partial opening of TJs and free passage through injured endothelium [252]. If the method will be proved safe and effective in more experimental models, MRI-guided focused ultrasound may have a great potential to treat CNS malignancies [226].

Although the 0.02 m<sup>2</sup> surface area of the blood-cerebrospinal fluid barrier created by the choroid plexus is negligible compared to the surface area of the BBB, it plays an important role in the regulation of drug transport and metabolism in the CNS [253,254]. Blood vessels in the choroid plexus are leaky, and the barrier is formed by epithelial cells expressing TJ proteins occludin, claudin-1, -2, -5, -11, ZO-1 [255,256]. Modulation of TJ permeability in choroid plexus epithelium and at the BBB shows similarities. The barrier permeability is increased by cyclic AMP [257], and decreased by phorbol ester-induced activation of PKC both *in vitro* and *in vivo* [255,258,259]. The PKC-mediated permeabilization of epithelial cells is accompanied by dephosphorylation and reduced immunostaining of occludin, down-regulation of claudin-2, decreased immunoreactivity for claudin-2, -5, cadherin and  $\beta$ -catenin [255,258]. Modulation of TJs at the level of the choroid plexus might provide an additional route for drug delivery to the CNS.

## 7. Concluding remarks

In contrast to strategies targeting transepithelial/transendothelial pathways, modulators acting on TJs could enhance paracellular permeability of biological barriers for a large number and variety of drugs, including hydrophilic compounds, biopharmaceuticals, like peptides, proteins, nucleic acids, and viral vectors without the need to modify the drugs. The concept is proven by the clinical application of several AEs, like sodium caprate, cyclodextrins or oleic acid, which act, at least partly, through modification of epithelial tight junctions. Because of the low selectivity and relatively high toxicity of AEs there is a need for new TJ modulators.

Intense research in several laboratories to develop safe and effective TJ modulators will result in increasing number of new peptides specific for unique TJ proteins with better organ or region selectivity and lower toxicity in the near future. For example, the selectivity of C-CPE peptide targeting claudin-4 and acting in the jejunum, but not in colon, seems to be promising [117]. Targeting claudin-5, or claudin-1 could lead to TJ modulators selective for the BBB or for the skin.

Beside peptides, antibodies, antibody fragments and small interfering nucleic acids specific for TJ proteins could also be potential modulators of paracellular permeability. While signalling pathways regulating TJs look promising targets of chemical compound modulators, like kinase inhibitors, they are far from being clinically applicable. The organ or tissue selectivity and the safety of these molecules have to be first proven.

Opening of TJs for a drug molecule might result in the enhanced transport of other molecules, xenobiotics or pathogens across the protective barriers of the GI tract, the BBB or the airways. TJs and their components play a fundamental role in morphogenesis and cell polarity. Safety assessment of TJ modulators should include careful testing and analysis for global adverse drug reactions, enhanced sensitivity for bacterial or viral infections, and long-term carcinogenic or teratogenic effects.

Some of the new TJ modulators have already entered clinical trials. AT1001 (Table 2), a TJ modulator to decrease intestinal hyperpermeability, has been tested for safety and efficacy to treat celiac disease [260]. The effect of the permeability enhancer PN-159 is being examined in Phase II studies on nasal insulin and peptide YY<sub>3–36</sub> administration to control type 2 diabetes and induce weight loss in obesity, respectively [261].

As a conclusion, the number of TJ modulators with a potential to be used in drug delivery is expected to greatly increase in the near future and provide an unprecedented selectivity for various barriers, as well as better efficacy and safety.

## Acknowledgements

This work was supported by grants from the National Office for Research and Technology (RET 08/2004) and the Hungarian Ministry of Health ETT 589/2006. The help of Dr. Csongor Ábrahám for critical reading of the manuscript is gratefully acknowledged.

## References

- [1] L. González-Mariscal, P. Nava, Tight junctions, from tight intercellular seals to sophisticated protein complexes involved in drug delivery, pathogens interaction and cell proliferation, *Adv. Drug Deliv. Rev.* 57 (2005) 811–814.
- [2] H. Chiba, M. Osanai, M. Murata, T. Kojima, N. Sawada, Transmembrane proteins of tight junctions, *Biochim. Biophys. Acta* 1778 (2008) 729–756.
- [3] L. González-Mariscal, R. Tapia, D. Chamorro, Crosstalk of tight junction components with signalling pathways, *Biochim. Biophys. Acta* 1778 (2008) 729–756.
- [4] M. Takano, R. Yumoto, T. Murakami, Expression and function of efflux drug transporters in the intestine, *Pharmacol. Ther.* 109 (2006) 137–161.
- [5] N.J. Abbott, L. Rönnback, E. Hansson, Astrocyte-endothelial interactions at the blood–brain barrier, *Nat. Rev. Neurosci.* 7 (2006) 41–53.

- [6] R.S. El-Bacha, A. Minn, Drug metabolizing enzymes in cerebrovascular endothelial cells afford a metabolic protection to the brain, *Cell. Mol. Biol.* 45 (1999) 15–23 (Noisy-le-grand).
- [7] P.D. Ward, T.K. Tippin, D.R. Thakker, Enhancing paracellular permeability by modulating epithelial tight junctions, *Pharm. Sci. Technol. Today* 3 (2000) 346–358.
- [8] Y. Masaoka, Y. Tanaka, M. Kataoka, S. Sakuma, S. Yamashita, Site of drug absorption after oral administration: assessment of membrane permeability and luminal concentration of drugs in each segment of gastrointestinal tract, *Eur. J. Pharm. Sci.* 29 (2006) 240–250.
- [9] G. Scheuch, M.J. Kohlhaeufel, P. Brand, R. Siekmeier, Clinical perspectives on pulmonary systemic and macromolecular delivery, *Adv. Drug Deliv. Rev.* 58 (2006) 996–1008.
- [10] A. Naik, Y.N. Kalia, R.H. Guy, Transdermal drug delivery: overcoming the skin's barrier function, *Pharm. Sci. Technol. Today* 3 (2000) 318–326.
- [11] R. Cecchelli, V. Berezowski, S. Lundquist, M. Culot, M. Renftel, M.-P. Dehouck, L. Fénart, Modelling of the blood–brain barrier in drug discovery and development, *Nat. Rev. Drug Discov.* 6 (2007) 650–661.
- [12] H. Wolburg, A. Lippoldt, Tight junctions of the blood–brain barrier: development, composition and regulation, *Vasc. Pharmacol.* 38 (2002) 323–337.
- [13] N.J. Abbott, Astrocyte–endothelial interactions and blood–brain barrier permeability, *J. Anat.* 200 (2002) 629–638.
- [14] W.M. Pardridge, Drug and gene targeting to brain with molecular Trojan horses, *Nat. Rev. Drug Discov.* 1 (2002) 131–139.
- [15] E. Neuwelt, N.J. Abbott, L. Abrey, W.A. Banks, B. Blakley, T. Davis, B. Engelhardt, P. Grammas, M. Nedergaard, J. Nutt, W. Pardridge, G.A. Rosenberg, Q. Smith, L.R. Drewes, Strategies to advance translational research into brain barriers, *Lancet Neurol.* 7 (2008) 84–96.
- [16] L. Illum, Nasal drug delivery-possibilities, problems and solutions, *J. Control. Release* 87 (2003) 187–198.
- [17] H. Wolburg, K. Wolburg-Buchholz, H. Sam, S. Horvát, M.A. Deli, A.F. Mack, Epithelial and endothelial barriers in the olfactory region of the nasal cavity of the rat, *Histochem. Cell Biol.* 130 (2008) 127–140.
- [18] L. Illum, Nasal drug delivery: new developments and strategies, *Drug Discov. Today* 7 (2002) 1184–1189.
- [19] M. Abdulrazik, S. Tamilvanan, S. Benita, Non-systemic delivery of topical brimonidine to the brain: a neuro-ocular tissue distribution study, *J. Drug Target.* 15 (2006) 670–679.
- [20] A. Lambiase, L. Pagani, V. Di Fausto, V. Sposato, M. Coassin, S. Bonini, L. Aloe, Nerve growth factor eye drop administered on the ocular surface of rodents affects the nucleus basalis and septum: biochemical and structural evidence, *Brain Res.* 1127 (2007) 45–51.
- [21] G. Krause, L. Winkler, S.L. Mueller, R.F. Haseloff, J. Piontek, I.E. Blasig, Structure and function of claudins, *Biochim. Biophys. Acta* 1778 (2008) 631–645.
- [22] L. Paris, L. Tonutti, C. Vannini, G. Bazzoni, Structural organization of the tight junctions, *Biochim. Biophys. Acta* 1778 (2008) 646–659.
- [23] A.W. Vorbrodt, D.H. Dobrogowska, Molecular anatomy of interendothelial junctions in human blood–brain barrier microvessels, *Folia Histochem. Cytobiol.* 42 (2004) 67–75.
- [24] A. Nusrat, C.A. Parkos, P. Verkade, C.S. Foley, T.W. Liang, W. Innis-Whitehouse, K. K. Eastburn, J.L. Madara, Tight junctions are membrane microdomains, *J. Cell Sci.* 113 (2000) 1771–1781.
- [25] M. Furuse, T. Hirase, M. Itoh, A. Nagafuchi, S. Yonemura, S. Tsukita, S. Tsukita, Occludin: a novel integral membrane protein localizing at tight junctions, *J. Cell Biol.* 123 (1993) 1777–1788.
- [26] M. Saitou, M. Furuse, H. Sasaki, J.D. Schulzke, M. Fromm, H. Takano, T. Noda, S. Tsukita, Complex phenotype of mice lacking occludin, a component of tight junction strands, *Mol. Biol. Cell* 11 (2000) 4131–4142.
- [27] D. Yu, J.R. Turner, Stimulus-induced reorganization of tight junction structure: the role of membrane traffic, *Biochim. Biophys. Acta* 1778 (2008) 709–716.
- [28] J. Ikenouchi, M. Furuse, K. Furuse, H. Sasaki, S. Tsukita, S. Tsukita, Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells, *J. Cell Biol.* 171 (2005) 939–945.
- [29] S. Riazuddin, Z.M. Ahmed, A.S. Fanning, A. Lagziel, S. Kitajiri, K. Ramzan, S.N. Khan, P. Chatteraj, P.L. Friedman, J.M. Anderson, I.A. Belyantseva, A. Forge, S. Riazuddin, T.B. Friedman, Tricellulin is a tight-junction protein necessary for hearing, *Am. J. Hum. Genet.* 79 (2006) 1040–1051.
- [30] T.A. Martin, Loss of barrier function and its role in cancer metastasis, *Biochim. Biophys. Acta* (2008–this issue).
- [31] V. Tasic, D. Dervisov, S. Kocova, S. Weber, M. Konrad, Hypomagnesemia with hypercalciuria and nephrocalcinosis: case report and a family study, *Pediatr. Nephrol.* 20 (2005) 1003–1006.
- [32] M. Konrad, A. Schaller, D. Seelow, A.V. Pandey, S. Waldegger, A. Lesslauer, H. Vitzthum, Y. Suzuki, J.M. Luk, C. Becker, K.P. Schlingmann, M. Schmid, J. Rodriguez-Soriano, G. Ariceta, F. Cano, R. Enriquez, H. Juppner, S.A. Bakkaloglu, M.A. Hediger, S. Gallati, S.C. Neuhaus, P. Nurnberg, S. Weber, Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement, *Am. J. Hum. Genet.* 79 (2006) 949–957.
- [33] J.H. Lipschutz, S. Li, A. Arisco, D.F. Balkovetz, Extracellular signal-regulated kinases 1/2 control claudin-2 expression in Madin–Darby canine kidney strain I and II cells, *J. Biol. Chem.* 280 (2005) 3780–3788.
- [34] M. Furuse, M. Hata, K. Furuse, Y. Yoshida, A. Haratake, Y. Sugitani, T. Noda, A. Kubo, S. Tsukita, Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1 deficient mice, *J. Cell Biol.* 156 (2002) 1099–1111.
- [35] K.M. McCarthy, I.B. Skare, M.C. Stankewich, M. Furuse, S. Tsukita, R.A. Rogers, R.D. Lynch, E.E. Schneeberger, Occludin is a functional component of the tight junction, *J. Cell Sci.* 109 (1996) 2287–2298.
- [36] A. Tamura, Y. Kitano, M. Hata, T. Katsumo, K. Moriwaki, H. Sasaki, H. Hayashi, Y. Suzuki, T. Noda, M. Furuse, S. Tsukita, S. Tsukita, Megaintestine in claudin-15-deficient mice, *Gastroenterology* 134 (2008) 523–534.
- [37] T. Nitta, M. Hata, S. Gotoh, Y. Seo, H. Sasaki, N. Hashimoto, M. Furuse, S. Tsukita, Size-selective loosening of the blood–brain barrier in claudin-5-deficient mice, *J. Cell Biol.* 161 (2003) 653–660.
- [38] K. Hirata, T. Ishida, K. Penta, M. Rezaee, E. Yang, J. Wohlgemuth, T. Quertermous, Cloning of an immunoglobulin family adhesion molecule selectively expressed by endothelial cells, *J. Biol. Chem.* 276 (2001) 16223–16231.
- [39] W.M. Pardridge, Blood–brain barrier genomics, *Stroke* 38 (Suppl 2) (2007) 686–690.
- [40] F. Wegmann, B. Petri, A.G. Khandoga, C. Moser, A. Khandoga, S. Volkery, H. Li, I. Nasdala, O. Brandau, R. Fässler, S. Butz, F. Krombach, D. Vestweber, ESAM supports neutrophil extravasation, activation of Rho, and VEGF-induced vascular permeability, *J. Exp. Med.* 203 (2006) 1671–1677.
- [41] B.B. Finlay, Tight junction proteins as targets of infectious agents, *Biochim. Biophys. Acta* (2008–this issue).
- [42] M. Kondoh, A. Takahashi, M. Fuji, K. Yagi, Y. Watanabe, A novel strategy for a drug delivery system using a claudin modulator, *Biol. Pharm. Bull.* 29 (2006) 1783–1789.
- [43] M. Kondoh, K. Yagi, Tight junction modulators: promising candidates for drug delivery, *Curr. Med. Chem.* 14 (2007) 2482–2488.
- [44] A. Hartsock, W.J. Nelson, Adherens and tight junctions: structure, function and, connections to the actin cytoskeleton, *Biochim. Biophys. Acta* 1778 (2008) 660–669.
- [45] D. Pal, K.L. Audus, T.J. Siahaan, Modulation of cellular adhesion in bovine brain microvessel endothelial cells by a decapeptide, *Brain Res.* 747 (1997) 103–113.
- [46] J.M. Halbleib, W.J. Nelson, Cadherins in development: cell adhesion, sorting, and tissue morphogenesis, *Genes Dev.* 20 (2006) 3199–3214.
- [47] J.A. Tunggal, I. Helfrich, A. Schmitz, H. Schwarz, D. Gunzel, M. Fromm, R. Kemler, T. Krieg, C.M. Niessen, E-cadherin is essential for in vivo epidermal barrier function by regulating tight junctions, *EMBO J.* 24 (2005) 1146–1156.
- [48] J. Zabner, M. Winter, K.J. Excoffon, D. Stoltz, D. Ries, S. Shasby, M. Shasby, Histamine alters E-cadherin cell adhesion to increase human airway epithelial permeability, *J. Appl. Physiol.* 95 (2003) 394–401.
- [49] M. Conacci-Sorrell, J. Zhurinsky, A. Ben-Ze'ev, The cadherin–catenin adhesion system in signaling and cancer, *J. Clin. Invest.* 109 (2002) 987–991.
- [50] E. Dejana, M.G. Lampugnani, O. Martinez-Estrada, G. Bazzoni, The molecular organization of endothelial junctions and their functional role in vascular morphogenesis and permeability, *Int. J. Dev. Biol.* 44 (2000) 743–748.
- [51] M.T. Broman, D. Mehta, A.B. Malik, Cdc42 regulates the restoration of endothelial adherens junctions and permeability, *Trends Cardiovasc. Med.* 17 (2007) 151–156.
- [52] J. Gavard, J.S. Gutkind, VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin, *Nat. Cell Biol.* 8 (2006) 1223–1234.
- [53] C.M. Niessen, Tight junctions/adherens junctions: basic structure and function, *J. Invest. Dermatol.* 127 (2007) 2525–2532.
- [54] K. Irie, K. Shimizu, T. Sakisaka, W. Ikeda, Y. Takai, Roles and modes of action of nectins in cell–cell adhesion, *Semin. Cell Dev. Biol.* 15 (2004) 643–656.
- [55] S. Fabre-Lafay, F. Monville, S. Garrido-Urbani, C. Berruyer-Pouyet, C. Ginestier, N. Raymond, P. Finetti, R. Sauvan, J. Adélaïde, J. Geneix, E. Lecocq, C. Popovici, P. Dubreuil, P. Viens, A. Gonçalves, E. Charafe-Jauffret, J. Jacquemier, D. Birnbaum, M. Lopez, Nectin-4 is a new histological and serological tumor associated marker for breast cancer, *BMC Cancer* 7 (2007) 73.
- [56] A.R. Schenkel, T.W. Chew, W.A. Muller, Platelet endothelial cell adhesion molecule deficiency or blockade significantly reduces leukocyte emigration in a majority of mouse strains, *J. Immunol.* 173 (2004) 6403–6408.
- [57] Z. Sun, K. Olanders, A. Lasson, M. Dib, M. Annborn, K. Andersson, X. Wang, R. Andersson, Effective treatment of gut barrier dysfunction using an antioxidant, a PAF inhibitor, and monoclonal antibodies against the adhesion molecule PECAM-1, *J. Surg. Res.* 105 (2002) 220–233.
- [58] J.P. Couty, C. Rampon, M. Leveque, M.P. Laran-Chich, S. Bourdoulous, J. Greenwood, P.O. Couraud, PECAM-1 engagement counteracts ICAM-1-induced signaling in brain vascular endothelial cells, *J. Neurochem.* 103 (2007) 793–801.
- [59] M. Pentecost, G. Otto, J.A. Theriot, M.R. Amieva, *Listeria monocytogenes* invades the epithelial junctions at sites of cell extrusion, *PLoS Pathogens* 2 (2006) 29–40.
- [60] Z. Yu, S. Li, Y.Y. Huang, Y. Fong, R.J. Wong, Calcium depletion enhances nectin-1 expression and herpes oncolytic therapy of squamous cell carcinoma, *Cancer Gene Ther.* 14 (2007) 738–747.
- [61] K. Matter, M.S. Balda, Functional analysis of tight junctions, *Methods* 30 (2003) 228–234.
- [62] I.A. Krizbai, M.A. Deli, Signalling pathways regulating the tight junction permeability in the blood–brain barrier, *Cell. Mol. Biol.* 49 (2003) 23–31 (Noisy-Le-Grand).
- [63] H. Clarke, N. Ginanni, K.V. Laughlin, J.B. Smith, G.R. Pettit, J.M. Mullin, The transient increase of tight junction permeability can result from protein kinase C activation/translocation and act as a tumor promotional event in epithelial cancers, *Exp. Cell Res.* 261 (2000) 239–249.
- [64] M.A. Deli, M.P. Dehouck, C.S. Abraham, R. Cecchelli, F. Joó, Penetration of small molecular weight substances through cultured bovine brain capillary endothelial cell monolayers: the early effects of 3',5'-cyclic adenosine monophosphate, *Exp. Physiol.* 80 (1995) 675–678.



- [65] B. Kis, M.A. Deli, H. Kobayashi, C.S. Ábrahám, T. Yanagita, H. Kaiya, T. Isse, R. Nishi, S. Gotoh, K. Kangawa, A. Wada, J. Greenwood, M. Niwa, H. Yamashita, Y. Ueta, Adrenomedullin regulates blood–brain barrier functions *in vitro*, *Neuroreport* 12 (2001) 4139–4142.
- [66] M.A. Deli, C.S. Ábrahám, Y. Kataoka, M. Niwa, Permeability studies on *in vitro* blood–brain barrier models: physiology, pathology and pharmacology, *Cell. Mol. Neurobiol.* 25 (2005) 59–127.
- [67] M. Honda, S. Nakagawa, K. Hayashi, N. Kitagawa, K. Tsutsumi, I. Nagata, M. Niwa, Adrenomedullin improves the blood–brain barrier function through the expression of claudin-5, *Cell. Mol. Neurobiol.* 26 (2006) 109–118.
- [68] J.B. Mackic, M. Stins, S. Jovanovic, K.S. Kim, R.T. Bartus, B.V. Zlokovic, Cereport (RMP-7) increases the permeability of human brain microvascular endothelial cell monolayers, *Pharm. Res.* 16 (1999) 1360–1365.
- [69] T. Hirase, S. Kawashima, E.Y. Wong, T. Ueyama, Y. Rikitake, S. Tsukita, M. Yokoyama, J.M. Staddon, Regulation of tight junction permeability and occludin phosphorylation by RhoA-p16OROCK-dependent and -independent mechanisms, *J. Biol. Chem.* 276 (2001) 10423–10431.
- [70] K. Umeda, J. Ikenouchi, S. Katahira-Tayama, K. Furuse, H. Sasaki, M. Nakayama, T. Matsui, S. Tsukita, M. Furuse, S. Tsukita, ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation, *Cell* 126 (2006) 741–754.
- [71] M. Bruewer, A.M. Hopkins, M.E. Hobert, A. Nusrat, J.L. Madara, RhoA, Rac1, and Cdc42 exert distinct effects on epithelial barrier via selective structural and biochemical modulation of junctional proteins and F-actin, *Am. J. Physiol. Cell Physiol.* 287 (2004) C327–C335.
- [72] G. Schreibelt, G. Kooij, A. Reijkerker, R. van Doorn, S.I. Gringhuis, S. van der Pol, B.B. Weksler, I.A. Romero, P.O. Couraud, J. Piontek, I.E. Blasig, C.D. Dijkstra, E. Ronken, H.E. de Vries, Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling, *FASEB J.* 21 (2007) 3666–3676.
- [73] O.N. El-Assal, G.E. Besner, HB-EGF enhances restitution after intestinal ischemia/reperfusion via PI3K/Akt and MEK/ERK1/2 activation, *Gastroenterology* 129 (2005) 609–625.
- [74] S. Fischer, M. Wiesnet, D. Renz, W. Schaper, H<sub>2</sub>O<sub>2</sub> induces paracellular permeability of porcine brain-derived microvascular endothelial cells by activation of the p44/42 MAP kinase pathway, *Eur. J. Cell Biol.* 84 (2005) 687–697.
- [75] C. Vogel, A. Bauer, M. Wiesnet, K.T. Preissner, W. Schaper, H.H. Marti, S. Fischer, Flt-1, but not Flk-1 mediates hyperpermeability through activation of the PI3-K/Akt pathway, *J. Cell. Physiol.* 212 (2007) 236–243.
- [76] D. Langford, R. Hurford, M. Hashimoto, M. Digicaylioglu, E. Masliah, Signalling crosstalk in FGF2-mediated protection of endothelial cells from HIV-gp120, *BMC Neurosci.* 6 (2005) 8.
- [77] I.E. András, H. Pu, J. Tian, M.A. Deli, A. Nath, B. Hennig, M. Toborek, Signaling mechanisms of HIV-1 Tat-induced alterations of claudin-5 expression in brain endothelial cells, *J. Cereb. Blood Flow Metab.* 25 (2005) 1159–1170.
- [78] K. Grebenkämper, H.J. Galla, Translational diffusion measurements of a fluorescent phospholipid between MDCK-I cells support the lipid model of the tight junctions, *Chem. Phys. Lipids* 71 (1994) 133–143.
- [79] C.A. Feltkamp, A.W. Van der Waerden, Junction formation between cultured normal rat hepatocytes. An ultrastructural study on the presence of cholesterol and the structure of developing tight-junction strands, *J. Cell. Sci.* 63 (1983) 271–286.
- [80] M.C. Stankewich, S.A. Francis, Q.U. Vu, E.E. Schneeberger, R.D. Lynch, Alterations in cell cholesterol content modulate Ca<sup>2+</sup>-induced tight junction assembly by MDCK cells, *Lipids* 31 (1996) 817–828.
- [81] D. Lambert, C.A. O'Neill, P.J. Padfield, Methyl-beta-cyclodextrin increases permeability of Caco-2 cell monolayers by displacing specific claudins from cholesterol rich domains associated with tight junctions, *Cell. Physiol. Biochem.* 20 (2007) 495–506.
- [82] S.A. Francis, J.M. Kelly, J. McCormack, R.A. Rogers, J. Lai, E.E. Schneeberger, R.D. Lynch, Rapid reduction of MDCK cell cholesterol by methyl-β-cyclodextrin alters steady-state transepithelial electrical resistance, *Eur. J. Cell Biol.* 78 (1999) 473–484.
- [83] P.H. Johnson, S.C. Quay, Advances in nasal drug delivery through tight junction technology, *Expert Opin. Drug Deliv.* 2 (2005) 281–298.
- [84] M. Kondoh, T. Yoshida, H. Kakutani, K. Yagi, Targeting tight junction proteins – significance for drug development, *Drug Discov. Today* 13 (2008) 180–186.
- [85] P.H. Johnson, D. Frank, H.R. Constantino, Discovery of tight junction modulators: significance for drug development and therapy, *Drug Discov. Today* 13 (2008) 261–267.
- [86] V. Wong, B. Gumbiner, A synthetic peptide corresponding to the extracellular domain of occludin perturbs the tight junction permeability barrier, *J. Cell Biol.* 136 (1997) 399–409.
- [87] F. Lacaz-Vieira, M.M. Jaeger, P. Farshori, B. Kachar, Small synthetic peptides homologous to segments of the first external loop of occludin impair tight junction resealing, *J. Membr. Biol.* 168 (1999) 289–297.
- [88] S. Tavelin, K. Hashimoto, J. Malkinson, L. Lazorova, I. Toth, P. Artursson, A new principle for tight junction modulation based on occludin peptides, *Mol. Pharmacol.* 64 (2003) 1530–1540.
- [89] R.S. Everett, M.K. Vanhook, N. Barozzi, I. Toth, L.G. Johnson, Specific modulation of airway epithelial tight junctions by apical application of an occludin peptide, *Mol. Pharmacol.* 69 (2006) 492–500.
- [90] C.H. Wong, D.D. Mruk, W.M. Lee, C.Y. Cheng, Targeted and reversible disruption of the blood–testis barrier by an FSH mutant-occludin peptide conjugate, *FASEB J.* 21 (2007) 438–448.
- [91] S.C. Quay, Composition for enhanced epithelial permeation of peptide YY for treating obesity, United States patent Application, Pub. No.: US 2007/0275893 A1 (2007).
- [92] H. Wan, H.L. Winton, C. Soeller, D.C. Gruenert, P.I. Thompson, M.B. Cannell, G.A. Stewart, D.R. Garrod, C. Robinson, Quantitative structural and biochemical analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1, *Clin. Exp. Allergy* 30 (2000) 685–698.
- [93] D.J. O'Mahony, G. Cagney, Composition and method for enhancing paracellular transport across cell layers, United States Patent, Patent No.: US 6,346,613 B1 (2002).
- [94] K. Cui, B.H. Dutzar, Method for opening tight junctions, United States Patent Application, Pub. No.: US 2007/0253956 A1 (2007).
- [95] N. Sonoda, M. Furuse, H. Sasaki, S. Yonemura, J. Katahira, Y. Horiguchi, S. Tsukita, *Clostridium perfringens* enterotoxin fragment removes specific claudins from tight junction strands: evidence for direct involvement of claudins in tight junction barrier, *J. Cell Biol.* 147 (1999) 195–204.
- [96] T.A. Bird, A. Youakim, Claudin polypeptides, polynucleotides, and methods of making and use thereof, United States Patent Application Publication, Pub. No.: US 2008/0009004 (2008).
- [97] E. Sinaga, D.S.S. Jois, M. Avery, I.T. Makagiansar, U.S.F. Tambunan, K.L. Audus, T.J. Siahaan, Increasing paracellular porosity by E-cadherin peptides: discovery of bulge and groove regions in the EC1-domain of E-cadherin, *Pharm. Res.* 19 (2002) 1170–1179.
- [98] A. Fasano, B. Baudry, D.W. Pumphlin, S.S. Wasserman, B.D. Tall, J.M. Ketley, J.B. Kaper, *Vibrio cholerae* produces a second enterotoxin, which affects intestinal tight junctions, *Proc. Natl. Acad. Sci. U. S. A.* 88 (1991) 5242–5246.
- [99] A. Fasano, C. Fiorentini, G. Donelli, S. Uzzau, J.B. Kaper, K. Margarettin, X. Ding, S. Guandalini, L. Comstock, S.E. Goldblum, Zonula occludens toxin modulates tight junctions through protein kinase C-dependent actin reorganization, *in vitro*, *J. Clin. Invest.* 96 (1995) 710–720.
- [100] A. Fasano, S. Uzzau, Modulation of intestinal tight junctions by zonula occludens toxin permits enteral administration of insulin and other macromolecules in an animal model, *J. Clin. Invest.* 99 (1997) 1158–1164.
- [101] M. DiPiero, R. Lu, S. Uzzau, W. Wang, K. Margarettin, C. Pazzani, F. Maimone, A. Fasano, Zonula occludens toxin structure–function analysis. Identification of the fragment biologically active on tight junctions and of the zonulin receptor binding domain, *J. Biol. Chem.* 276 (2001) 19160–19165.
- [102] T. Watts, I. Berti, A. Sapone, T. Gerarduzzi, T. Not, R. Zielke, A. Fasano, Role of the intestinal tight junction modulator zonulin in the pathogenesis of type 1 diabetes in BB diabetic-prone rats, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 2916–2921.
- [103] A. Fasano, B. Paterson, Use of tight junction agonists to facilitate pulmonary delivery of therapeutic agents, United States Patent Application, Pub. No.: US 2008/0014218 A1 (2008).
- [104] K. Cui, S.-C. Chen, M.E. Houston, S.C. Quay, Tight junction modulator peptide PN159 for enhanced mucosal delivery of therapeutic compounds, United States Patent Application, Pub. No.: US 2006/0062758 A1 (2006).
- [105] K. Cui, S.-C. Chen, K.T. Fry, Tight junction modulating peptides for enhanced mucosal delivery of therapeutic compounds, United States Patent Application, Pub. No.: US 2007/0154449 A1 (2007).
- [106] A. Chandrasekaran, S. Sengupta, D.A. Berry, K. Holley, G. Zhao, R. Sasisekharan, Methods and compositions related to the modulation of extracellular tight junctions, United States Patent Application Publication, Pub. No.: US 2006/0067927 A1 (2006).
- [107] R.E. Herman, E.K. Makienko, M.G. Prieve, M. Fuller, M.E. Houston, P.H. Johnson, Phage display screening of epithelial cell monolayers treated with EGTA: identification of peptide FDFWTP that modulates tight junction activity, *J. Biomol. Screen.* 12 (2007) 1092–1101.
- [108] T. Oshima, O. Blaschuk, B. Gour, M. Symonds, J.W. Elrod, M. Sasaki, T.H. Jackson, J.S. Alexander, Tight junction peptide antagonists enhance neutrophil trans-endothelial chemotaxis, *Life Sci.* 73 (2003) 1729–1740.
- [109] A. Masuyama, M. Kondoh, H. Seguchi, A. Takahashi, M. Harada, M. Fujii, H. Mizuguchi, Y. Horiguchi, Y. Watanabe, Role of N-terminal amino acids in the absorption-enhancing effects of the c-terminal fragment of *Clostridium perfringens* enterotoxin, *J. Pharmacol. Exp. Ther.* 314 (2005) 789–795.
- [110] A. Takahashi, M. Kondoh, A. Masuyama, M. Fujii, H. Mizuguchi, Y. Horiguchi, Y. Watanabe, Role of C-terminal regions of the C-terminal fragment of *Clostridium perfringens* enterotoxin in its interaction with claudin-4, *J. Control. Release* 108 (2005) 56–62.
- [111] E. Sinaga, D.S.S. Jois, M. Avery, I.T. Makagiansar, U.S.F. Tambunan, K.L. Audus, T.J. Siahaan, Increasing paracellular porosity by E-cadherin peptides: discovery of bulge and groove regions in the EC1-domain of E-cadherin, *Pharm. Res.* 19 (2002) 1170–1179.
- [112] N.D. Eddington, A. Fasano, K.-H. Song, Oral delivery of therapeutic agents using tight junction agonists, United States Patent Application, Pub. No. US 2007/0196272 A1 (2007).
- [113] W. Wang, S. Uzzau, S.E. Goldblum, A. Fasano, Human zonulin, a potential modulator of intestinal tight junctions, *J. Cell. Sci.* 113 (2000) 4435–4440.
- [114] I. Vietor, T. Bader, K. Paiha, L.A. Huber, Perturbation of the tight junction permeability barrier by occludin loop peptides activates β-catenin/TCF/LEF-mediated transcription, *EMBO Reports* 2 (2001) 306–312.
- [115] N.N. Salama, A. Fasano, M. Thakar, N.D. Eddington, The impact of ΔG on the oral bioavailability of low bioavailable therapeutic agents, *J. Pharmacol. Exp. Ther.* 312 (2005) 199–205.
- [116] K.-H. Song, A. Fasano, N.D. Eddington, Effect of the six-mer synthetic peptide



- (AT1002) fragment of zonula occludens toxin on the intestinal absorption of cyclosporin A, *Int. J. Pharm.* 351 (2008) 8–14.
- [117] M. Kondoh, A. Masuyama, A. Takahashi, N. Asano, H. Mizuguchi, N. Koizumi, M. Fujii, T. Hayakawa, Y. Horiguchi, Y. Watanabe, A novel strategy for the enhancement of drug absorption using a claudin modulator, *Mol. Pharmacol.* 67 (2005) 749–756.
- [118] Z. Shao, Y. Li, T. Chermak, A.K. Mitra, Cyclodextrins as mucosal absorption promoters of insulin. II. Effects of beta-cyclodextrin derivatives on alpha-chymotryptic degradation and enteral absorption of insulin in rats, *Pharm. Res.* 11 (1994) 1174–1179.
- [119] A. Yamamoto, H. Tatsumi, M. Maruyama, T. Uchiyama, N. Okada, T. Fujita, Modulation of intestinal permeability by nitric oxide donors: implications in intestinal delivery of poorly absorbable drugs, *J. Pharmacol. Exp. Ther.* 296 (2001) 84–90.
- [120] G. Fetihi, F. Habib, N. Okada, T. Fujita, M. Attia, A. Yamamoto, Nitric oxide donors can enhance the intestinal transport and absorption of insulin and [Asu(1,7)]-eel calcitonin in rats, *J. Control. Release* 106 (2005) 287–297.
- [121] G. Fetihi, F. Habib, H. Katsumi, N. Okada, T. Fujita, M. Attia, A. Yamamoto, Excellent absorption enhancing characteristics of NO donors for improving the intestinal absorption of poorly absorbable compound compared with conventional absorption enhancers, *Drug Metab. Pharmacokinet.* 21 (2006) 222–229.
- [122] J.D. Söderholm, H. Oman, L. Blomquist, J. Veen, T. Lindmark, G. Olaison, Reversible increase in tight junction permeability to macromolecules in rat ileal mucosa by sodium caprate, a constituent of milk fat, *Dig. Dis. Sci.* 43 (1998) 1547–1552.
- [123] D. Cox, H. Gao, S. Raje, K. Scott, N.D. Eddington, Enhancing the permeation of marker compounds and emantinone anticonvulsants across Caco-2 monolayers by modulating tight junctions using zonula occludens toxin, *Eur. J. Pharm. Biopharm.* 52 (2001) 145–150.
- [124] D. Cox, S. Raje, H. Gao, N.N. Salama, N.D. Eddington, Enhanced permeability of molecular weight markers and poorly bioavailable compounds across Caco-2 monolayers using the absorption enhancer, zonula occludens toxin, *Pharm. Res.* 19 (2002) 1680–1688.
- [125] M. Tomita, M. Hayashi, S. Awazu, Comparison of absorption-enhancing effect between sodium caprate and disodium ethylenediaminetetraacetate in Caco-2 cells, *Biol. Pharm. Bull.* 17 (1994) 753–755.
- [126] Y.S. Quan, T. Fujita, F. Kamiyama, A. Yamamoto, Electrophysiological studies on the evaluation of absorption enhancers in Caco-2 cells using a microelectrode technique, *Biol. Pharm. Bull.* 23 (2000) 738–742.
- [127] T. Sawai, R.A. Drongowski, R.W. Lampman, A.G. Coran, C.M. Harmon, The effect of phospholipids and fatty acids on tight-junction permeability and bacterial translocation, *Pediatr. Surg. Int.* 17 (2001) 269–274.
- [128] B. Aspenström-Fagerlund, L. Ring, P. Aspenström, J. Tallkvist, N.G. Ilbäck, A.W. Glynn, Oleic acid and docosahexaenoic acid cause an increase in the paracellular absorption of hydrophilic compounds in an experimental model of human absorptive enterocytes, *Toxicology* 237 (2007) 12–23.
- [129] T. Lindmark, T. Nikkilä, P. Artursson, Mechanisms of absorption enhancement by medium chain fatty acids in intestinal epithelial Caco-2 cell monolayers, *J. Pharmacol. Exp. Ther.* 275 (1995) 958–964.
- [130] T. Lindmark, Y. Kimura, P. Artursson, Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells, *J. Pharmacol. Exp. Ther.* 284 (1998) 362–369.
- [131] E.L. Lecluyse, S.C. Sutton, J.A. Fix, In vitro effects of long-chain acylcarnitines on the permeability, transepithelial electrical resistance and morphology of rat colonic mucosa, *J. Pharmacol. Exp. Ther.* 265 (1993) 955–962.
- [132] J.H. Hochman, J.A. Fix, E.L. Lecluyse, In vitro and in vivo analysis of the mechanism of absorption enhancement by palmitoylcarnitine, *J. Pharmacol. Exp. Ther.* 269 (1994) 813–822.
- [133] E.K. Anderberg, C. Nyström, P. Artursson, Epithelial transport of drugs in cell culture. VII: Effects of pharmaceutical surfactant excipients and bile acids on transepithelial permeability in monolayers of human intestinal epithelial (Caco-2) cells, *J. Pharm. Sci.* 81 (1992) 79–87.
- [134] E.K. Anderberg, T. Lindmark, P. Artursson, Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route, *Pharm. Res.* 10 (1993) 857–864.
- [135] D. Dimitrijevic, A.J. Shaw, A.T. Florence, Effects of some non-ionic surfactants on transepithelial permeability in Caco-2 cells, *J. Pharm. Pharmacol.* 52 (2000) 157–162.
- [136] M.H. Doo, H. Li, H.I. Jang, I.S. Song, S.J. Chung, C.K. Shim, Effect of nonylphenol ethoxylates (NPEs) on barrier functions of epithelial cell membranes: opening of tight junctions and competitive inhibition of P-gp-mediated efflux, *Int. J. Pharm.* 302 (2005) 145–153.
- [137] G. Ranaldi, I. Marigliano, I. Vespignani, G. Perozzi, Y. Sambuy, The effect of chitosan and other polycations on tight junction permeability in the human intestinal Caco-2 cell line, *J. Nutr. Biochem.* 13 (2002) 157–167.
- [138] J.M. Smith, M. Dornish, E.J. Wood, Involvement of protein kinase C in chitosan glutamate-mediated tight junction disruption, *Biomaterials* 26 (2005) 3269–3276.
- [139] M. Marinaro, A. Fasano, M.T. De Magistris, Zonula occludens toxin acts as an adjuvant through different mucosal routes and induces protective immune responses, *Infect. Immun.* 71 (2003) 1897–1902.
- [140] T. Lindmark, J.D. Söderholm, G. Olaison, G. Alván, G. Ocklind, P. Artursson, Mechanisms of absorption enhancement in humans after rectal administration of ampicillin in suppositories containing sodium caprate, *Pharm. Res.* 14 (1997) 930–935.
- [141] Y. Watanabe, Y. Matsumoto, M. Seki, M. Takase, M. Matsumoto, Absorption enhancement of polypeptide drugs by cyclodextrins. I. Enhanced rectal absorption of insulin from hollow-type suppositories containing insulin and cyclodextrins in rabbits, *Chem. Pharm. Bull. (Tokyo)* 40 (1992) 3042–3047.
- [142] N. Utoguchi, Y. Watanabe, T. Shida, M. Matsumoto, Nitric oxide donors enhance rectal absorption of macromolecules in rabbits, *Pharm. Res.* 15 (1998) 870–876.
- [143] M. Rossi, F. Maurano, D. Luongo, A. Fasano, S. Uzzau, S. Auricchio, R. Troncone, Zonula occludens toxin (Zot) interferes with the induction of nasal tolerance to gliadin, *Immunol. Lett.* 81 (2002) 217–221.
- [144] K.-H. Song, A. Fasano, N.D. Eddington, Enhanced nasal absorption of hydrophilic markers after dosing with AT1002, a tight junction modulator, *Eur. J. Pharm. Biopharm.* 69 (2008) 231–237.
- [145] S.C. Chen, K. Eiting, K. Cui, A.K. Leonard, D. Morris, C.Y. Li, K. Farber, A.P. Sileno, M.E. Houston, P.H. Johnson, S.C. Quay, H.R. Constantino, Therapeutic utility of a novel tight junction modulating peptide for enhancing intranasal drug delivery, *J. Pharm. Sci.* 95 (2006) 1364–1371.
- [146] G. Wang, J. Zabner, C. Deering, J. Launspach, J. Shao, M. Bodner, D.J. Jolly, B.L. Davidson, P.B. McCray, Increasing epithelial junction permeability enhances gene transfer to airway epithelia *in vivo*, *Am. J. Respir. Cell Mol. Biol.* 22 (2000) 129–138.
- [147] H. Lin, M. Gebhardt, S. Bian, K.A. Kwon, C.K. Shim, S.J. Chung, D.D. Kim, Enhancing effect of surfactants on fexofenadine.HCl transport across the human nasal epithelial cell monolayer, *Int. J. Pharm.* 330 (2007) 23–31.
- [148] Y. Watanabe, Y. Matsumoto, K. Kawamoto, S. Yazawa, M. Matsumoto, Enhancing effect of cyclodextrins on nasal absorption of insulin and its duration in rabbits, *Chem. Pharm. Bull. (Tokyo)* 40 (1992) 3100–3104.
- [149] T. Yang, A. Hussain, J. Paulson, T.J. Abbruscato, F. Ahsan, Cyclodextrins in nasal delivery of low-molecular-weight heparins: *in vivo* and *in vitro* studies, *Pharm. Res.* 21 (2004) 1127–1136.
- [150] C.A. Herbert, C.M. King, P.C. Ring, S.T. Holgate, G.A. Stewart, P.J. Thompson, C. Robinson, Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p1, *Am. J. Respir. Cell Mol. Biol.* 12 (1995) 369–378.
- [151] C.B. Coyne, M.M. Kelly, R.C. Boucher, L.G. Johnson, Enhanced epithelial gene transfer by modulation of tight junctions with sodium caprate, *Am. J. Respir. Cell Mol. Biol.* 23 (2000) 602–609.
- [152] L.Y. Wang, J.K. Ma, W.F. Pan, D. Toledo-Velasquez, C.J. Malanga, Y. Rojanasakul, Alveolar permeability enhancement by oleic acid and related fatty acids: evidence for a calcium-dependent mechanism, *Pharm. Res.* 11 (1994) 513–517.
- [153] K. Morita, H. Sasaki, M. Furuse, S. Tsukita, Endothelial claudin: claudin-5/TMVC constitutes tight junction strands in endothelial cells, *J. Cell Biol.* 147 (1999) 185–194.
- [154] K. Fujita, J. Katahira, Y. Horiguchi, N. Sonoda, M. Furuse, S. Tsukita, *Clostridium perfringens* enterotoxin binds to the second extracellular loop of claudin-3, a tight junction integral membrane protein, *FEBS Lett.* 476 (2000) 258–261.
- [155] R. Lu, W. Wang, S. Uzzau, R. Vigorito, H.R. Zielke, A. Fasano, Affinity purification and partial characterization of the zonulin/zonula occludens toxin (Zot) receptor from human brain, *J. Neurochem.* 74 (2000) 320–326.
- [156] N.N. Salama, N.D. Eddington, A. Fasano, Tight junction modulation and its relationship to drug delivery, *Adv. Drug Deliv. Rev.* 58 (2006) 15–28.
- [157] W.L. Wang, R.L. Lu, M. DiPierro, A. Fasano, Zonula occludin toxin, a microtubule binding protein, *World J. Gastroenterol.* 6 (2000) 330–334.
- [158] C.S. Karyekar, A. Fasano, S. Raje, R. Lu, T.C. Dowling, N.D. Eddington, Zonula occludens toxin increases the permeability of molecular weight markers and chemotherapeutic agents across the bovine brain microvessel endothelial cells, *J. Pharm. Sci.* 92 (2003) 414–423.
- [159] M. Tomita, M. Hayashi, S. Awazu, Absorption-enhancing mechanism of EDTA, caprate, and decanoylcarnitine in Caco-2 cells, *J. Pharm. Sci.* 85 (1996) 608–611.
- [160] R. Notman, M.G. Noro, J. Anwar, Interaction of oleic acid with dipalmitoylphosphatidylcholine (DPPC) bilayers simulated by molecular dynamics, *J. Phys. Chem. B.* 111 (2007) 12748–12755.
- [161] J.Y. Park, Y.M. Kim, H.S. Song, K.Y. Park, Y.M. Kim, M.S. Kim, Y.K. Pak, I.K. Lee, J.D. Lee, S.J. Park, K.U. Lee, Oleic acid induces endothelin-1 expression through activation of protein kinase C and NF-kappa B, *Biochem. Biophys. Res. Commun.* 303 (2003) 891–895.
- [162] S.Y. Oh, B.V. Madhukar, J.E. Trosko, Inhibition of gap junctional blockage by palmitoyl carnitine and TMB-8 in a rat liver epithelial cell line, *Carcinogenesis* 9 (1988) 135–139.
- [163] C. Schulze, C. Smales, L.L. Rubin, J.M. Staddon, Lysophosphatidic acid increases tight junction permeability in cultured brain endothelial cells, *J. Neurochem.* 68 (1997) 991–1000.
- [164] T. Chiu, E. Rozengurt, PKD in intestinal epithelial cells: rapid activation by phorbol esters, LPA, and angiotensin through PKC, *Am. J. Physiol. Cell. Physiol.* 280 (2001) C929–C942.
- [165] K.P. Xu, X.F. Li, F.S. Yu, Corneal organ culture model for assessing epithelial responses to surfactants, *Toxicol. Sci.* 58 (2000) 306–314.
- [166] K. Dharmasathaphorn, P.A. Huott, P. Vongkovit, G. Beuerlein, S.J. Pandol, H.V. Ammon, Cl<sup>−</sup> secretion induced by bile salts. A study of the mechanism of action based on a cultured colonic epithelial cell line, *J. Clin. Invest.* 84 (1989) 945–953.
- [167] Z. Nagy, H. Peters, I. Hüttner, Charge-related alterations of the cerebral endothelium, *Lab. Invest.* 49 (1983) 662–671.
- [168] E.B. Peixoto, C.B. Collares-Buzato, Protamine-induced epithelial barrier disruption involves rearrangement of cytoskeleton and decreased tight junction-associated protein expression in cultured MDCK strains, *Cell Struct. Funct.* 29 (2005) 165–178.
- [169] S. Vepa, W.M. Scribner, V. Natarajan, Activation of endothelial cell phospholipase D by polycations, *Am. J. Physiol.* 272 (1997) L608–L613.
- [170] N.G. Schipper, S. Olsson, J.A. Hoogstraate, A.G. de Boer, K.M. Vårum, P. Artursson, Chitosan as absorption enhancers for poorly absorbable drugs 2: mechanism of absorption enhancement, *Pharm. Res.* 14 (1997) 923–929.

- [171] V. Dodane, M. Amin Khan, J.R. Merwin, Effect of chitosan on epithelial permeability and structure, *Int. J. Pharm.* 182 (1999) 21–32.
- [172] V. Monnaert, D. Betbeder, L. Fenart, H. Bricout, A.M. Lenfant, C. Landry, R. Cecchelli, E. Monflier, S. Tilloy, Effects of gamma- and hydroxypropyl-gamma-cyclodextrins on the transport of doxorubicin across an in vitro model of blood–brain barrier, *J. Pharmacol. Exp. Ther.* 311 (2004) 1115–1120.
- [173] V. Monnaert, S. Tilloy, H. Bricout, L. Fenart, R. Cecchelli, E. Monflier, Behavior of alpha-, beta-, and gamma-cyclodextrins and their derivatives on an in vitro model of blood–brain barrier, *J. Pharmacol. Exp. Ther.* 310 (2004) 745–751.
- [174] N.S. Ningaraj, M. Rao, K. Hashizume, K. Asotra, K.L. Black, Regulation of blood–brain tumor barrier permeability by calcium-activated potassium channels, *J. Pharmacol. Exp. Ther.* 301 (2002) 838–851.
- [175] A. Farkas, E. Szatmári, A. Orbók, I. Wilhelm, K. Wejksza, P. Nagyösi, P. Hutamekalin, H. Bauer, H.-C. Bauer, A. Traweger, I.A. Krizbai, Hyperosmotic mannitol induces Src kinase-dependent phosphorylation of beta-catenin in cerebral endothelial cells, *J. Neurosci. Res.* 80 (2005) 855–861.
- [176] S.B. Raymond, J. Skoch, K. Hynynen, B.J. Bacskai, Multiphoton imaging of ultrasound/Optison mediated cerebrovascular effects in vivo, *J. Cereb. Blood Flow Metab.* 27 (2007) 393–403.
- [177] J.A. Nicolazzo, B.L. Reed, B. Finnin, Buccal penetration enhancers – how do they really work? *J. Control. Release* 105 (2005) 1–15.
- [178] Y.S. Quan, K. Hattori, E. Lundborg, T. Fujita, M. Murakami, S. Muranishi, A. Yamamoto, Effectiveness and toxicity screening of various absorption enhancers using Caco-2 cell monolayers, *Biol. Pharm. Bull.* 21 (1998) 615–620.
- [179] A. Kogan, N. Garti, Microemulsions as transdermal drug delivery vehicles, *Adv. Colloid Interface Sci.* 123–126 (2006) 369–385.
- [180] A. Melero, T.M. Garrigues, P. Almudever, A.M. Villodre, C.M. Lehr, U. Schäfer, Nortriptyline hydrochloride skin absorption: development of a transdermal patch, *Eur. J. Pharm. Biopharm.* 69 (2008) 588–596.
- [181] L. Sztrihai, A.L. Betz, Oleic acid reversibly opens the blood–brain barrier, *Brain Res.* 550 (1991) 257–262.
- [182] H.J. Kim, Y.S. Pyeun, Y.W. Kim, B.M. Cho, T.H. Lee, T.Y. Moon, K.T. Suh, B.R. Park, A model for research on the blood–brain barrier disruption induced by unsaturated fatty acid emulsion, *Invest. Radiol.* 40 (2005) 270–276.
- [183] T. Lindmark, N. Schipper, L. Lazorová, A.G. de Boer, P. Artursson, Absorption enhancement in intestinal epithelial Caco-2 cell monolayers by sodium caprate: assessment of molecular weight dependence and demonstration of transport routes, *J. Drug Target.* 5 (1998) 215–223.
- [184] E. Duizer, C. van der Wulp, C.H. Versantvoort, J.P. Groten, Absorption enhancement, structural changes in tight junctions and cytotoxicity caused by palmitoyl carnitine in Caco-2 and IEC-18 cells, *J. Pharmacol. Exp. Ther.* 287 (1998) 395–402.
- [185] R.G. Strickley, Solubilizing excipients in oral and injectable formulations, *Pharm. Res.* 21 (2004) 201–230.
- [186] S.S. Davis, L. Illum, Absorption enhancers for nasal drug delivery, *Clin. Pharmacokinet.* 42 (2003) 1107–1128.
- [187] M. Suzuki, M. Machida, K. Adachi, K. Otabe, T. Sugimoto, M. Hayashi, S. Awazu, Histopathological study of the effects of a single intratracheal instillation of surface active agents on lung in rats, *J. Toxicol. Sci.* 25 (2000) 49–55.
- [188] G.T.A. McEwan, M.A. Jepson, B.H. Hirst, N.L. Simmons, Polycation-induced enhancement of epithelial paracellular permeability is independent of tight junctional characteristics, *Biochim. Biophys. Acta* 1148 (1993) 51–60.
- [189] X.Y. Yu, B.H. Schofield, T. Croxton, N. Takahashi, E.W. Gabrielson, E.W. Spannhake, Physiologic modulation of bronchial epithelial cell barrier function by polycationic exposure, *Am. J. Respir. Cell Mol. Biol.* 11 (1994) 188–198.
- [190] C. Valenta, B.G. Auner, The use of polymers for dermal and transdermal delivery, *Eur. J. Pharm. Biopharm.* 58 (2004) 279–289.
- [191] M. Thanou, J.C. Verhoef, H.E. Junginger, Oral drug absorption enhancement by chitosan and its derivatives, *Adv. Drug Deliv. Rev.* 52 (2001) 117–126.
- [192] H.O. Alpar, S. Somavarapu, K.N. Atuah, V.W. Bramwell, Biodegradable mucoadhesive particulates for nasal and pulmonary antigen and DNA delivery, *Adv. Drug Deliv. Rev.* 57 (2005) 411–430.
- [193] J.M. Smith, E.J. Wood, M. Dornish, Effect of chitosan on epithelial cell tight junctions, *Pharm. Res.* 21 (2004) 43–49.
- [194] T. Loftsson, S.B. Vogensen, M.E. Brewster, F. Konráðsdóttir, Effects of cyclodextrins on drug delivery through biological membranes, *J. Pharm. Sci.* 96 (2007) 2532–2546.
- [195] F.W. Merkus, J.C. Verhoef, S.G. Romeijn, N.G. Schipper, Absorption enhancing effect of cyclodextrins on intranasally administered insulin in rats, *Pharm. Res.* 8 (1991) 588–592.
- [196] T. Kiss, F. Fenyvesi, B.I. Kovácsné, P. Fehér, K.R. Leposáné, J. Váradi, L. Szenté, E. Fenyvesi, R. Iványi, M. Vecsernyés, Cytotoxic examinations of various cyclodextrin derivatives on Caco-2 cells, *Acta Pharm. Hung.* 77 (2007) 150–154.
- [197] L. Matilainen, T. Toropainen, H. Vihola, J. Hirvonen, T. Järvinen, P. Jarho, K. Järvinen, In vitro toxicity and permeation of cyclodextrins in Calu-3 cells, *J. Control. Release* 126 (2008) 10–16.
- [198] M.P. Fink, R.L. Delude, Epithelial barrier dysfunction: a unifying theme to explain the pathogenesis of multiple organ dysfunction at the cellular level, *Crit. Care Clin.* 21 (2005) 177–196.
- [199] X. Han, M.P. Fink, R. Yang, R.L. Delude, Increased iNOS activity is essential for intestinal epithelial tight junction dysfunction in endotoxemic mice, *Shock* 21 (2004) 261–270.
- [200] N. Numata, K. Takahashi, N. Mizuno, N. Utoguchi, Y. Watanabe, M. Matsumoto, T. Mayumi, Improvement of intestinal absorption of macromolecules by nitric oxide donor, *J. Pharm. Sci.* 89 (2000) 1296–1304.
- [201] Y. Watanabe, N. Utoguchi, A. Ishii, Y. Tai, H. Shiota, H. Kurai, M. Matsumoto, Absorption enhancement of a protein drug by nitric oxide donor: effect on nasal absorption of human granulocyte colony-stimulating factor, *J. Drug Target.* 8 (2000) 185–194.
- [202] K. Takahashi, N. Numata, N. Kinoshita, N. Utoguchi, T. Mayumi, N. Mizuno, Characterization of the influence of nitric oxide donors on intestinal absorption of macromolecules, *Int. J. Pharm.* 286 (2004) 89–97.
- [203] D. Menon, C.S. Karyekar, A. Fasano, R. Lu, N.D. Eddington, Enhancement of brain distribution of anticancer agents using  $\Delta G$ , the 12 kDa active fragment of ZOT, *Int. J. Pharm.* 306 (2005) 122–131.
- [204] K. Nagasawa, H. Chiba, H. Fujita, T. Kojima, T. Saito, T. Endo, N. Sawada, Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells, *J. Cell. Physiol.* 208 (2006) 123–132.
- [205] D.F. Emerich, P. Snodgrass, M. Pink, F. Bloom, R.T. Bartus, Central analgesic actions of loperamide following transient permeation of the blood–brain barrier with Cereport (RMP-7), *Brain Res.* 801 (1998) 252–266.
- [206] C.V. Borlongan, D.F. Emerich, B.J. Hoffer, R.T. Bartus, Bradykinin receptor agonist facilitates low-dose cyclosporin-A protection against 6-hydroxydopamine neurotoxicity, *Brain Res.* 956 (2002) 211–220.
- [207] C.V. Borlongan, D.F. Emerich, Facilitation of drug entry into the CNS via transient permeation of blood–brain barrier: laboratory and preliminary clinical evidence from bradykinin receptor agonist, *Cereport, Brain Res. Bull.* 60 (2003) 297–306.
- [208] D.F. Emerich, R.L. Dean, C. Osborn, R.T. Bartus, The development of the bradykinin agonist labradimil as a means to increase the permeability of the blood–brain barrier: from concept to clinical evaluation, *Clin. Pharmacokinet.* 40 (2001) 105–123.
- [209] K. Warren, R. Jalecki, B. Widemann, A. Aikin, M. Libucha, R. Packer, G. Vezina, G. Reaman, D. Shaw, M. Krailo, C. Osborne, J. Cehelsky, D. Caldwell, J. Stanwood, S.M. Steinberg, F.M. Balis, Phase II trial of intravenous lobaridimil and carboplatin in childhood brain tumors: a report from the Children's Oncology Group, *Cancer Chemother. Pharmacol.* 58 (2006) 343–347.
- [210] T. Nomura, K. Ikezaki, K. Matsukado, M. Fukui, Effect of histamine on the blood–tumor barrier in transplanted rat brain tumors, *Acta Neurochir. Suppl. (Wien)* 60 (1994) 400–402.
- [211] T. Inamura, T. Nomura, K. Ikezaki, M. Fukui, G. Pöllinger, K.L. Black, Intracarotid histamine infusion increases blood tumour permeability in RG2 glioma, *Neurol. Res.* 16 (1994) 125–128.
- [212] T.J. Raub, S.L. Kuentzel, G.A. Sawada, Permeability of bovine brain microvessel endothelial cells *in vitro*: barrier tightening by a factor released from astrogloma cells, *Exp. Cell Res.* 199 (1992) 330–340.
- [213] E. Preston, J. Slinn, I. Vinokourov, D. Stanimirovic, Graded reversible opening of the rat blood–brain barrier by intracarotid infusion of sodium caprate, *J. Neurosci. Methods* 168 (2008) 443–449.
- [214] A. Saija, P. Princi, D. Trombetta, M. Lanza, A. De Pasquale, Changes in the permeability of the blood–brain barrier following sodium dodecyl sulphate administration in the rat, *Exp. Brain Res.* 115 (1997) 546–551.
- [215] M.K. Spigelman, R.A. Zappulla, L.I. Malis, J.F. Holland, S.J. Goldsmith, J.D. Goldberg, Intracarotid dehydrocholate infusion: a new method for prolonged reversible blood–brain barrier disruption, *Neurosurgery* 12 (1983) 606–612.
- [216] R.A. Zappulla, M.K. Spigelman, E. Omsberg, J.J. Rosen, L.I. Malis, J.F. Holland, Electroencephalographic consequences of sodium dehydrocholate-induced blood–brain barrier disruption: Part I. Acute and chronic effects of intracarotid sodium dehydrocholate, *Neurosurgery* 16 (1985) 630–638.
- [217] J. Greenwood, J. Adu, A.J. Davey, N.J. Abbott, M.W.B. Bradbury, The effect of bile salts on the permeability and ultrastructure of the perfused, energy-depleted, rat blood–brain barrier, *J. Cereb. Blood Flow Metab.* 11 (1991) 644–654.
- [218] M. Mikov, S. Kevresan, K. Kuhajda, V. Jakovljević, V. Vasović, 3-Alpha,7-alpha-dihydroxy-12-oxo-5beta-cholanate as blood–brain barrier permeator, *Pol. J. Pharmacol.* 56 (2004) 367–371.
- [219] I. Westergren, B.B. Johansson, Altering the blood–brain barrier in the rat by intracarotid infusion of polycations: a comparison between protamine, poly-L-lysine and poly-L-arginine, *Acta Physiol. Scand.* 149 (1993) 99–104.
- [220] J. Yao, J.P. Zhou, Q.N. Ping, Y. Lu, L. Chen, Distribution of nobilite chitosan-based microemulsions in brain following i.v. injection in mice, *Int. J. Pharm.* 352 (2008) 256–262.
- [221] S. Tilloy, V. Monnaert, L. Fenart, H. Bricout, R. Cecchelli, E. Monflier, Methylated beta-cyclodextrin as P-gp modulators for deliverance of doxorubicin across an in vitro model of blood–brain barrier, *Bioorg. Med. Chem. Lett.* 16 (2006) 2154–2157.
- [222] K.M. Boje, S.S. Lakhman, Nitric oxide redox species exert differential permeability effects on the blood–brain barrier, *J. Pharmacol. Exp. Ther.* 293 (2000) 545–550.
- [223] M.-P. Dehouck, S. Méresse, P. Delorme, J.C. Fruchart, R. Cecchelli, An easier, reproducible, and mass-production method to study the blood–brain barrier in vitro, *J. Neurochem.* 54 (1990) 1798–1801.
- [224] D.H. Dobrogowska, A.W. Vorbrod, Immunogold localization of tight junctional proteins in normal and osmotically-affected rat blood–brain barrier, *J. Mol. Histo.* 35 (2004) 529–539.
- [225] M.A. Deli, L. Descamps, M.-P. Dehouck, R. Cecchelli, F. Joó, C.S. Ábrahám, G. Torpier, Exposure of tumor necrosis factor  $\alpha$  to the luminal membrane induces a delayed increase of permeability and formation of cytoplasmic actin stress fibers in brain capillary endothelial cells cocultured with astrocytes, *J. Neurosci. Res.* 41 (1995) 717–726.
- [226] L.L. Muldoon, C. Soussain, K. Jahnke, C. Johanson, T. Siegal, Q.R. Smith, W.A. Hall, K. Hynynen, P.D. Senter, D.M. Peereboom, E.A. Neuwelt, Chemotherapy delivery issues in central nervous system malignancy: a reality check, *J. Clin. Oncol.* 25 (2007) 2295–2305.

- [227] B. Erdlenbruch, C. Schinkhof, W. Kugler, D.E.H. Heinemann, J. Herms, H. Eibl, M. Lakomek, Intracarotid administration of short-chain alkylglycerols for increased delivery of methotrexate to the brain, *Br. J. Pharmacol.* 139 (2003) 685–694.
- [228] B. Erdlenbruch, M. Alipour, G. Fricker, D.S. Miller, W. Kugler, H. Eibl, M. Lakomek, Alkylglycerol opening of the blood–brain barrier to small and large fluorescence markers in normal and C6 glioma-bearing rats and isolated rat brain capillaries, *Br. J. Pharmacol.* 140 (2003) 1201–1210.
- [229] K. Hynynen, N. McDannold, N. Vykhodtseva, S. Raymond, R. Weissleder, F.A. Jolesz, N. Sheikov, Focal disruption of the blood–brain barrier due to 260-kHz ultrasound bursts: a method for molecular imaging and targeted drug delivery, *J. Neurosurg.* 105 (2006) 445–454.
- [230] M. Kinoshita, N. McDannold, F. Jolesz, K. Hynynen, Targeted delivery of antibodies through the blood–brain barrier by MRI-guided focused ultrasound, *Biochem. Biophys. Res. Commun.* 340 (2006) 1085–1090.
- [231] M. Kinoshita, N. McDannold, F.A. Jolesz, K. Hynynen, Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood–brain barrier disruption, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 11719–11723.
- [232] L.H. Treat, N. McDannold, N. Vykhodtseva, Y. Zhang, K. Tam, K. Hynynen, Targeted delivery of doxorubicin to the rat brain at therapeutic levels using MRI-guided focused ultrasound, *Int. J. Cancer* 121 (2007) 901–907.
- [233] M.J. Williams, M.B. Lowrie, J.P. Bennett, J.A. Firth, P. Clark, Cadherin-10 is a novel blood–brain barrier adhesion molecule in human and mouse, *Brain Res.* 1058 (2005) 62–72.
- [234] B.J. Gentil, C. Benaud, C. Delphin, C. Remy, V. Berezowski, R. Cecchelli, O. Feraud, D. Vittet, J. Baudier, Specific AHNK expression in brain endothelial cells with barrier properties, *J. Cell. Physiol.* (2004) 133–144.
- [235] F. Joó, The role of histamine in brain oedema formation, *Funct. Neurology* 8 (1993) 243–250.
- [236] T.F. Cloughesy, K.L. Black, Pharmacological blood–brain barrier modification for selective drug delivery, *J. Neurooncol.* 26 (1995) 125–132.
- [237] P. Vajkoczy, M.D. Menger, Vascular microenvironment in gliomas, *Cancer Treat. Res.* 117 (2004) 249–262.
- [238] H. Ishihara, H. Kubota, R.L. Lindbergh, D. Leppert, S.M. Gloor, M. Errede, D. Virgintino, A. Fontana, Y. Yonekawa, K. Frei, Endothelial cell barrier impairment induced by glioblastomas and transforming growth factor beta2 involves matrix metalloproteinases and tight junction proteins, *J. Neuropathol. Exp. Neurol.* 67 (2008) 435–448.
- [239] S.R. Doctrow, S.M. Abelleira, L.A. Curry, R. Heller-Harrison, J.W. Kozarich, B. Malfroy, L.A. McCarroll, K.G. Morgan, A.R. Morrow, G.F. Musso, The bradykinin analog RMP-7 increases intracellular free calcium levels in rat brain microvascular endothelial cells, *J. Pharmacol. Exp. Ther.* 271 (1994) 229–237.
- [240] E. Sanovich, R.T. Bartus, P.M. Friden, R.L. Dean, H.Q. Lea, M.W. Brightman, Pathway across blood–brain barrier opened by the bradykinin agonist, RMP-7, *Brain Res.* 705 (1995) 125–135.
- [241] X. Zhang, Y. Xie, Y. Jin, X. Hou, L. Ye, J. Lou, The effect of RMP-7 and its derivative on transporting Evans blue liposomes into the brain, *Drug Deliv.* 11 (2004) 301–309.
- [242] M.H. Sarker, P.A. Fraser, The role of guanylyl cyclases in the permeability response to inflammatory mediators in pial venular capillaries in the rat, *J. Physiol.* 540 (2002) 209–218.
- [243] A. Yamauchi, S. Dohgu, T. Nishioku, H. Shuto, M. Naito, T. Tsuruo, Y. Sawada, Y. Kataoka, An inhibitory role of nitric oxide in the dynamic regulation of the blood–brain barrier function, *Cell. Mol. Neurobiol.* 27 (2007) 263–270.
- [244] T.W. Gardner, T. Leshner, S. Khin, C. Vu, A.J. Barber, W.A. Brennan Jr., Histamine reduces ZO-1 tight-junction protein expression in cultured retinal microvascular endothelial cells, *Biochem. J.* 320 (1996) 717–721.
- [245] T. Nitz, T. Eisenblätter, K. Psathaki, H.J. Galla, Serum-derived factors weaken the barrier properties of cultured porcine brain capillary endothelial cells in vitro, *Brain Res.* 981 (2003) 30–40.
- [246] R.A. Zappulla, M.K. Spigelman, J.J. Rosen, D. Marotta, L.I. Malis, J.F. Holland, Electroencephalographic consequences of sodium dehydrocholate-induced blood–brain barrier disruption: Part 2. Generation and propagation of spike activity after the topical application of sodium dehydrocholate, *Neurosurgery* 16 (1985) 639–643.
- [247] O. Tomkins, O. Friedman, S. Ivens, C. Reiffurth, S. Major, J.P. Dreier, U. Heinemann, A. Friedman, Blood–brain barrier disruption results in delayed functional and structural alterations in the rat neocortex, *Neurobiol. Dis.* 25 (2007) 367–377.
- [248] S.I. Rapoport, Advances in osmotic opening of the blood–brain barrier to enhance CNS chemotherapy, *Expert Opin. Investig. Drugs* 10 (2001) 1809–1818.
- [249] B. Erdlenbruch, V. Jendrosseck, H. Eibl, M. Lakomek, Transient and controllable opening of the blood–brain barrier to cytostatic and antibiotic agents by alkylglycerols in rats, *Exp. Brain Res.* 135 (2000) 417–422.
- [250] B. Erdlenbruch, V. Jendrosseck, W. Kugler, H. Eibl, M. Lakomek, Increased delivery of erucylphosphocholine to C6 gliomas by chemical opening of the blood–brain barrier using intracarotid pentylglycerol in rats, *Cancer Chemother. Pharmacol.* 50 (2002) 299–304.
- [251] M. Kinoshita, Targeted drug delivery to the brain using focused ultrasound, *Top. Magn. Reson. Imaging* 17 (2006) 209–215.
- [252] N. Sheikov, N. McDannold, N. Vykhodtseva, F. Jolesz, K. Hynynen, Cellular mechanisms of the blood–brain barrier opening induced by ultrasound in presence of microbubbles, *Ultrasound Med. Biol.* 30 (2004) 979–989.
- [253] N. Strazielle, S.T. Khuth, J.F. Ghersi-Egea, Detoxification systems, passive and specific transport for drugs at the blood-CSF barrier in normal and pathological situations, *Adv. Drug Deliv. Rev.* 56 (2004) 1717–1740.
- [254] C.E. Johanson, J.A. Duncan, E.G. Stopa, A. Baird, Enhanced prospects for drug delivery and brain targeting by the choroid plexus-CSF route, *Pharm. Res.* 22 (2005) 1011–1037.
- [255] A. Lippoldt, S. Liebner, B. Andbjør, H. Kalbacher, H. Wolburg, H. Haller, K. Fuxe, Organization of choroid plexus epithelial and endothelial cell tight junctions and regulation of claudin-1, -2 and -5 expression by protein kinase C, *Neuroreport* 11 (2000) 1427–1431.
- [256] H. Wolburg, K. Wolburg-Buchholz, S. Liebner, B. Engelhardt, Claudin-1, claudin-2 and claudin-11 are present in tight junctions of choroid plexus epithelium of the mouse, *Neurosci. Lett.* 307 (2001) 77–80.
- [257] M. Haselbach, J. Wegener, S. Decker, C. Engelbertz, H.J. Galla, Porcine choroid plexus epithelial cells in culture: regulation of barrier properties and transport processes, *Microsc. Res. Tech.* 52 (2001) 137–152.
- [258] A. Lippoldt, A. Jansson, U. Kniesel, B. Andbjør, A. Andersson, H. Wolburg, K. Fuxe, H. Haller, Phorbol ester induced changes in tight and adherens junctions in the choroid plexus epithelium and in the ependyma, *Brain Res.* 854 (2000) 197–206.
- [259] S. Angelow, P. Zeni, B. Höhn, H.J. Galla, Phorbol ester induced short- and long-term permeabilization of the blood-CSF barrier in vitro, *Brain Res.* 1063 (2005) 168–179.
- [260] References for clinical trials investigating the safety and efficacy of AT-1001 can be found at <http://www.clinicaltrials.gov> (NCT00386165, NCT00386490, NCT00492960, NCT00620451).
- [261] References for clinical trials investigating the safety and efficacy of PN-159 can be found at <http://www.clinicaltrials.gov> (NCT00362856, NCT00537420, NCT00624767).