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Research Article

The Effect of Capsaicin Derivatives on Tight-Junction Integrity and Permeability of Madin-Darby Canine Kidney Cells

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ABSTRACT

Capsaicin is known to interfere with tight junctions (TJs) of epithelial cells and therefore to enhance paracellular permeability of poorly absorbable drugs. However, due to its low water solubility, pungency, and cytotoxicity, its pharmacologic use is limited. In this study, we investigated the effect of capsaicin derivatives of synthetic (e.g., 10-hydroxy-N-(4-hydroxy-3-methoxybenzyl)decanamide, etc.) and natural (olvanil and dihydrocapsaicin) origin on Madin-Darby Canine Kidney–C7 cells. Impedance spectroscopy was used to determine the transepithelial electrical resistance and the capacitance. Permeability assays with fluorescein isothiocyanate–dextran were carried out to evaluate the impact on cell permeability. The results show that lipophilicity could play an important role for the interference with TJ and that the mechanism is independent from the ion channel TRPV-1 and hence on the flux of calcium into the cells. In summary, we synthesized 4 derivatives of capsaicin of lower lipophilicity and compared their properties with other well-known vanilloids. We show that these compounds are able to enhance the permeability of a hydrophilic macromolecule, by opening the TJ for a shorter time than capsaicin. This behavior is dependent on the lipophilicity of the molecule. Understanding of these phenomena may lead to better control of administration of therapeutic molecules.

Introduction

Capsaicin is known for its pungent taste and occurs in nature as a constituent of chili peppers. It stimulates the TRPV-1 receptor, a relatively nonselective calcium channel, which is responsible for the sensation of heat.[1] Capsaicin is known to have versatile therapeutic effects such as the treatment of chronic pain via the desensitization of afferent sensory neurons.[2] Other applications are the control of body temperature or as anti-obesity drug.[3,4] More recently, it has been discovered that capsaicin has a profound reversible opening effect on cellular tight junctions (TJs).[5] This rigid cell–connecting network of transmembrane proteins which is linked to the actin skeleton can be found in epithelial and endothelial tissues.[6] It serves as a protection mechanism to control the permeation of unknown compounds. Especially, hydrophilic macromolecular drugs (e.g., proteins, polysaccharide, polynucleotides, etc.) are often unable to cross this barrier.[7] The property to reversibly open TJ makes capsaicin a potential permeability enhancer for drugs with a poor bioavailability.[8-10] However, the use of capsaicin also entails several drawbacks associated to its strong pungency and known cytotoxicity.[11] The application of high doses is not tolerable, especially in case of sensitive administration routes such as the nasal or oral mucosae. Furthermore, its lipophilic character causes solubility difficulties when handling the compound in aqueous environments,[12] which complicates a precise administration. Our previous studies in Madin-Darby Canine Kidney (MDCK)-C7 cells monolayers have shown that capsaicin is able to open reversible TJ for a time span of >10 h even at lower concentrations (≤500 mM).[10] For a specific uptake of the delivered drug, an immediate cell response and a very short time of opening would be advantageous to reduce the permeation of undesired substances. Based on this, we reasoned that it would be appropriate to modify the structure of capsaicin to improve its properties for the desired application. In the past, various

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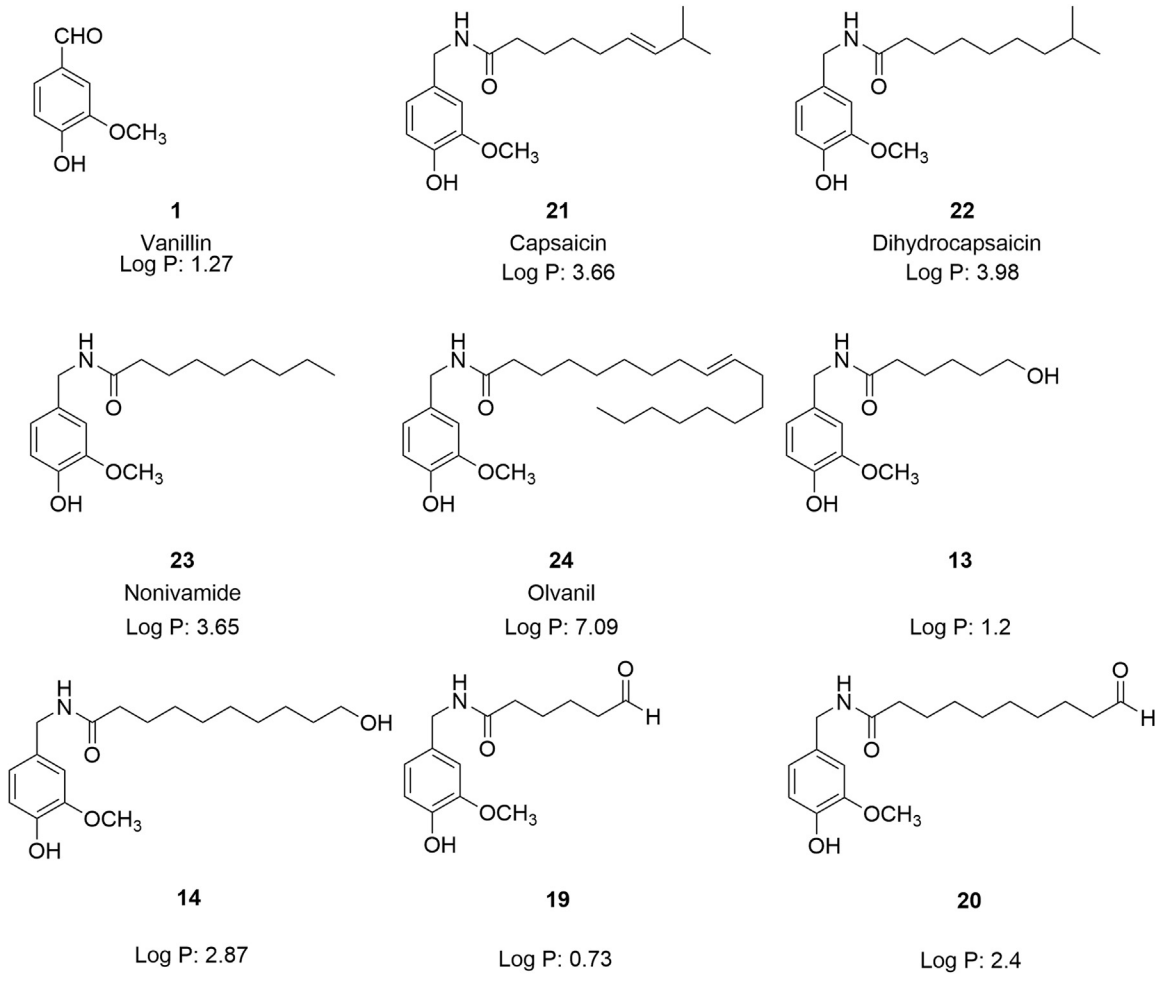

Figure 1. Vanilloids which have been used for this study. The theoretical Log *p* values were estimated.

capsaicin analogues have been synthesized mainly with the goal to find new agonists and antagonists.[13-15] Later, resiniferatoxin,[16] an agonist which is 3-4 orders of magnitude more potent than capsaicin, and capsazepine,[17] the first known antagonist, have been discovered. In addition, the structure–function relationship between different analogues and the TRPV-1 activation has been studied.[18-21] It has been found that different analogues induce varying profiles of calcium release inside cells[22] and furthermore that too high hydrophilicity or hydrophobicity reduces the pungency of a compound.[15,23] This can be explained by different permeability behaviors of compounds of different polarity through the cell membrane.[23] TRPV-1 receptors are also expressed in the endoplasmatic reticulum and can release calcium ions from internal storages.[18,24] In light of this, it has been hypothesized that different permeability behaviors influence also the release profile of intracellular calcium ions,[25] which could in turn also affect other responses of the cell. Our goal was to establish a comparison between molecular features of capsaicin derivatives of synthetic and natural origin and their biological activities in mammalian cells. In particular, we addressed the influence of these compounds on the paracellular transport of a model hydrophilic macromolecule (fluorescein isothiocyanate [FITC]–dextran). We aimed to shorten the duration of the TJ opening effect in comparison to capsaicin, thus facilitating a narrow time window of enhanced cell permeability. To this end, we synthesized 4 capsaicin analogues (13, 14, 19, 20) which have a low lipophilicity and also included capsaicin, vanillin, dihydrocapsaicin, nonivamide, and olvanil in the study (Fig. 1). We used the MDCK-C7 cell line as it is known to form a barrier of high integrity.[26] Impedance spectroscopy measurements, using a cellZscope® (nanoAnalytics, Münster, Germany) device[27] for online measurements, were performed to determine the transepithelial electrical resistance (TEER) and the capacitance (C_{CL}). To investigate permeation of a model macromolecule (FITC-dextran 4000 Da), cell permeability assays using were carried out. Furthermore, the influx of calcium into the cells was investigated. Our study shows that different derivatives of capsaicin modify the effect of reversible TJ opening and molecular transport.

Experimental Section

General Experimental Methods

All the chemicals were purchased from Spectrochem, Avra, Merck, and Sigma-Aldrich Chemical Companies and were of the highest purity. Capsaicin, dihydrocapsaicin, nonivamide, and olvanil were purchased from Sigma-Aldrich (Steinheim, Germany). The protocol of synthesis of the compounds 13, 14, 19, and 20 is described in the Supporting Information.

Cell Culture

MDCK-C7 cell line was cultured using minimal essential medium supplemented with 10% of fetal bovine serum, 1% of L-glutamine (200 mM), and 1% of penicillin–streptomycin (10,000