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BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

CD36- and GPR120-Mediated Ca^{2+} Signaling in Human Taste Bud Cells Mediates Differential Responses to Fatty Acids and Is Altered in Obese Mice

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BACKGROUND & AIMS: It is important to increase our understanding of gustatory detection of dietary fat and its contribution to fat preference. We studied the roles of the fat taste receptors CD36 and GPR120 and their interactions via Ca^{2+} signaling in fungiform taste bud cells (TBC). **METHODS:** We measured Ca^{2+} signaling in human TBC, transfected with small interfering RNAs against messenger RNAs encoding CD36 and GPR120 (or control small interfering RNAs). We also studied Ca^{2+} signaling in TBC from *CD36*$^{-/-}$ mice and from wild-type lean and obese mice. Additional studies were conducted with mouse enteroendocrine cell line STC-1 that express GPR120 and stably transfected with human *CD36*. We measured release of serotonin and glucagon-like peptide-1 from human and mice TBC in response to CD36 and GPR120 activation. **RESULTS:** High concentrations of linoleic acid induced Ca^{2+} signaling via CD36 and GPR120 in human and mice TBC, as well as in STC-1 cells, and low concentrations induced Ca^{2+} signaling via only CD36. Incubation of human and mice fungiform TBC with lineoleic acid down-regulated CD36 and up-regulated GPR120 in membrane lipid rafts. Obese mice had decreased spontaneous preference for fat. Fungiform TBC from obese mice had reduced Ca^{2+} and serotonin responses, but increased release of glucagon-like peptide-1, along with reduced levels of CD36 and increased levels of GPR120 in lipid rafts. **CONCLUSIONS:** CD36 and GPR120 have nonoverlapping roles in TBC signaling during orogustatory perception of dietary lipids; these are differentially regulated by obesity.

Keywords: Serotonin; Linoleic Acid; GLP-1; Lipids.

Oral perception of dietary fat was, until recently, thought to involve mainly texture and olfactory cues; however, accumulating evidence strongly suggests the existence of a taste modality devoted to the detection of long-chain fatty acids (LCFA).[1] Mice can recognize dietary fat and FA solutions in the oral cavity in the absence of olfactory or textural cues.[2] Humans can taste LCFA even when textural properties are masked and olfaction is eliminated using a nose clip.[2]

CD36 is highly expressed apically on lingual gustatory epithelium in rats and mice[3,4] and CD36 deletion completely abolishes the spontaneous preference of wild-type mice for LCFA.[4] In mouse circumvallate taste bud cells (TBC), LCFA-mediated activation of CD36 released Ca^{2+} from the endoplasmic reticulum (ER) via a phospholipase-C (PLC)–dependent mechanism.[5] In addition, LCFA via CD36 induced the phosphorylation of Src kinases, which regulate the opening of store-operated Ca^{2+} (SOC) channels in TBC. The SOC channels in the mouse TBC are composed of orai1/3 proteins, and are under the control of stromal interaction molecule-1, which orchestrates ER Ca^{2+} sensing and release.[6] Stromal interaction molecule-1 might play a key role in fat perception, as its deletion eliminates the mice's spontaneous preference for fat.[6]

Similar to CD36, the GPR120 and GPR40 members of G-protein–coupled receptors (GPCRs) were shown to mediate the taste responses to FAs.[7] Both GPR120 and GPR40 knock-out mice showed a diminished preference for linoleic and oleic acids with reduced taste nerve responses. GPR40 expression was undetectable in rat[8] and human TBC[9] and only GPR120, is expressed in type II cells, the true taste receptor cells of the lingual epithelium.[7]

The findings to date suggest that TBC CD36 and GPR120 are the potential candidates for lipid taste perception.[1,10] However, information on their relative roles remains largely unavailable. Why are there 2 receptor candidates for 1 taste?[11] There is evidence in mice that TBC CD36 and GPR120 might respond differently to dietary fat. During a meal, lingual CD36 expression is down-regulated, which is paralleled by reduction of fat preference.[12] In contrast, no changes are observed in

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Abbreviations used in this paper: **[Ca^{2+}]i, intracellular calcium; ER, endoplasmic reticulum; GA, grifolic acid; GLP-1, glucagon-like peptide-1; GPCRs, G protein-coupled receptors; HFD, high-fat diet; IP_3, inositol-tris-phosphate; LA, linoleic acid; LCFA, long-chain fatty acid; siRNA, small interfering RNA; SOC, store-operated Ca^{2+}; TBC, taste bud cells; WT, wild type.**

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GPR120 expression in circumvallate papillae during the meal,[12] suggesting that this receptor plays an alternative role distinct from that of CD36. The current study was undertaken to test the hypothesis that CD36, which has high affinity for LCFA, might function as the primary receptor for fat taste detection, and GPR120 would operate to amplify signaling mainly under excess FA supply. For this we elucidated Ca^{2+} signaling pathways, triggered by LCFA interaction with CD36 or GPR120 vs a specific agonist of GPR120 in human fungiform TBC. Selective small interfering RNA (siRNA) silencing of CD36 and GPR120 was used to dissect their respective roles. The data were further validated using TBC from *CD36*$^{-/-}$ mice and STC-1 endocrine cells, endogenously expressing GPR120 and transfected with human *CD36*. The effect of high-fat feeding on CD36 and GPR120 expression levels and membrane distribution were examined in fungiform TBC from lean and obese mice. Lipid gustatory preference, TBC Ca^{2+} signaling, and the associated release of serotonin and glucagon-like peptide-1 (GLP-1) were determined.

Materials and Methods

Materials

Human[13–15] and C57BL/6J mice TBC[5,6] were prepared and maintained as reported previously. All studies adhered to protocols approved by the Schulman Associates Institutional Review Board (Cincinnatti, OH) for human TBC and by the Regional Ethical Committee of Dijon (France) and the Animal Studies Committee of Washington University (St Louis, MO) for mice TBC. Cell culture media were from Lonza Verviers (Belgium) and Fura-2/AM from Invitrogen (Carlsbad, CA) (see Supplementary Materials).

Measurement of Ca^{2+} Signaling

Isolated TBC suspended in fresh Iscove's modified Dulbecco's medium containing 10% fetal bovine serum were seeded (2×10^5/well) onto Willico-Dish wells.[6] Changes in intracellular Ca^{2+} ($[Ca^{2+}]i$) were monitored using a Nikon microscope (TiU) equipped with EM-CCD (Luca-S) camera for real-time recording of digital images and an S-fluor 40× oil immersion objective (Nikon, Tokyo, Japan). Planes were taken at *Z* intervals of 0.3 μm and the images analyzed using NIS-Elements software (Nikon, Tokyo, Japan). Changes in $[Ca^{2+}]i$ were expressed as ΔRatio, calculated as the difference between the peak F_{340}/F_{380}. The data were averaged for 20−40 individual cells per run from 3−9 experiments with at least 3 cell preparations. For experiments in Ca^{2+}-free medium, $CaCl_2$ was replaced by ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (2 mM).

Statistical Analysis

Data are presented as mean ± SEM. Significance of differences between mean values was determined by one-way analysis of variance (Statistica, Statsoft, France), followed by a least-significant difference test.

Results

Human Fungiform TBC Co-express GPR120 and CD36

Our initial studies sought to determine the signaling events involved in transduction of fat taste in humans, with emphasis on the respective roles of CD36 and GPR120. To date, there is little information related to these processes in human TBC. Human fungiform TBC sorted for CD36 expression were found to express the markers of taste

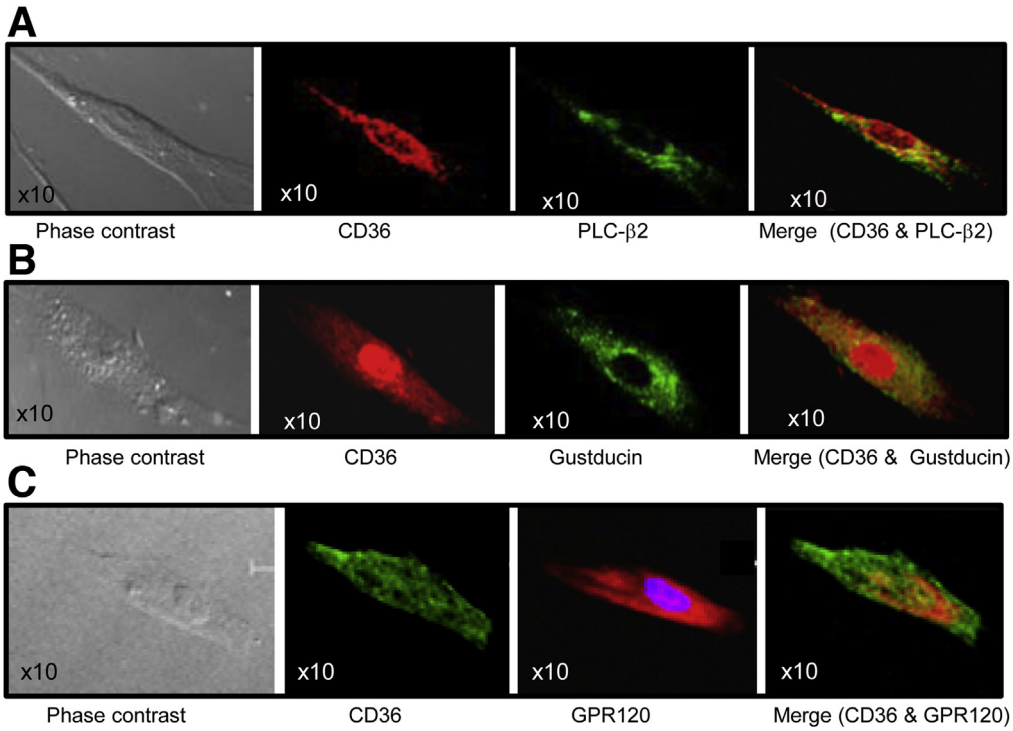

Figure 1. Characterization of human fungiform TBC. Images were acquired with Leica TCS-SP2 confocal laser scanning microscope (Leica, Buffalo Grove, IL) (*A*−*C*). Immunoreactivity for CD36 (*red* in *A*, *B*; *green* in *C*), GPR120 (*red* in *C*), and PLC-β (*green* in *A*) and α-gustducin (*green* in *B*) was observed in cultured TBC.