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Colocalization of GPR120 with phospholipase-Cβ2 and α-gustducin in the taste bud cells in mice

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ABSTRACT

A recent study has demonstrated that the G-protein coupled receptor GPR120 is expressed in the taste bud cells in rats. In this study, we have identified the types of taste cell that express GPR120 in C57/BL6 mice. Double immunostaining for GPR120 and the markers of type II taste cells (phospholipase-Cβ2 and α-gustducin) revealed that the majority of the GPR120-positive taste cells are type II taste cells. In contrast, it was observed that GPR120 was rarely colocalized with the marker of type III cells (neuronal cell adhesion molecule). These results suggested that GPR120 is mainly expressed in the type II taste cells and might function as a sensor for dietary fat.

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Several studies have indicated that rodents and humans recognize the presence of fat in foods not only by the texture of the food but also chemically in the mouth [7,9,10]: this suggests that the chemical perception of fatty acids is involved in the acquisition of a strong preference for fat. We recently reported that CD36, which is known as fatty acid translocase, is expressed in the taste bud cells in the tongue [5]. More recently, we reported that GPR120, a G-protein coupled receptor, is expressed in the rat taste bud cells [17]. GPR120, originally identified by Fredriksson by searching databases for sequences similar to rhodopsin-like GPRs [4], functions as a receptor for unsaturated long-chain fatty acids in the intestine [11]. Hirasawa demonstrated that unsaturated long-chain fatty acids, i.e., linoleic acid and oleic acid, increased intracellular Ca^{2+}, but the octanoic acid and fatty acid esters did not [11,13]. These results regarding the specificity of fatty acid derivatives are highly consistent with our previous data obtained in rats using the two-bottle choice test [24]. In addition, identical results regarding the specificity of fatty acids were found in the cephalic phase of pancreatic enzyme secretion induced by the ingestion of fatty acid derivatives in rats that were esophagotomized to prevent the stimulation of the gastrointestinal tract with the test substances [10]. Therefore, we concluded that GPR120 is a novel candidate for fatty acid receptors in the oral cavity.

Taste cells are divided into three types on the basis of their morphological and functional properties [21,22,25]. Type I cells are long spindle-shaped cells with several long microvilli, and they are known as "dark cells" because of their high cytoplasmic electron density; synaptic contacts are not observed among type I cells [19]. Type I cells express the glial glutamate/aspartate transporter [16], which indicates that these cells function like glial cells within taste buds.

Type II cells have all the components required for taste transduction, including receptors for umami, sweet, or bitter tastes along with downstream transduction components, and they function as receptor cells [1,2,8,12,20]. Only type III cells show synaptic conjunction, and can transmit taste signals to sensory afferent nerve fibers [3,14]. Therefore, type II and type III taste cells are involved in the transduction of gustartory stimuli. However, it is unclear which indicates of these cell types express GPR120. Therefore, in this study, we identified the type of cells that express GPR120 in mice by using double immunohistochemical staining in mice.

The lingual epithelia were separated from the connecting tissues by enzymatic dissociation (2.0 mg/mL type IV collagenase and elastase, and 1.0 mg/mL trypsin inhibitor), and the circumvallate papillae were dissected under a microscope. The dissected samples were rapidly homogenized by sonication in Sepasol-RNA I Super (Nacalai Tesque, Kyoto, Japan), and total RNA was extracted according to the manufacturer's instructions. Total RNA was then purified using PicoPure™ RNA Isolation Kit (Arcturus Bioscience, Mountain View, CA) and RNase-Free DNase Set (Qiagen,

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Hilden, Germany). cDNA was synthesized using 1 μg total RNA and Moloney murine leukemia virus reverse transcriptase (Promega Inc., Madison, WI). Polymerase chain reaction (PCR) was performed with 0.5 μM each of sense and antisense primers, and a thermal cycler (Takara PCR Thermal Cycler SP; Takara, Shiga, Japan). A glyceraldehydes-3-phosphate-dehydrogenase (GAPDH) transcript served as the internal control. The PCR conditions consisted of 30 cycles (for GPR120) and 23 cycles (for GAPDH); each cycle included 30 s of denaturation at 94 °C, 60 s of annealing at 55 °C, and a 60-s of extension at 72 °C. The sequences of the primers used for PCR are as follows: mouse GPR120, 5′-GCATAGGAGAAATCTCATGG-3′ and 5′-GAGTTGGCAAACGTGAAGGC-3′ (the expected sizes of the PCR products from mRNA, 340 bp); mouse GAPDH 5′-TGACCACAGTCCATGCCATC-3′ and 5′-TTGAAGTCGCAGGAGACAAC-3′ (expected size, 337 bp). The amplified cDNA was separated by electrophoresis on 2% agarose gels and stained with SYBR® Gold (Molecular Probes, Eugene, OR).

Mice were deeply anesthetized with sodium pentobarbital (100 mg/kg) and perfused via the aorta with 0.01 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (pH 7.4). The tongues were removed, and the circumvallate and fungiform papillae were excised. After fixation in the same fixative overnight, samples were immersed overnight in a 30% sucrose solution in 0.1 M PB and then frozen in liquid nitrogen. The samples were cut into 20-μm-thick sections with a cryostat and collected as they were free floating in 0.01 M PBS (pH 7.4). The sections were treated with 0.3% Triton X-100 in PBS for 2 h. After preincubation with Block Ace (Yukijirushi, Sapporo, Japan) for

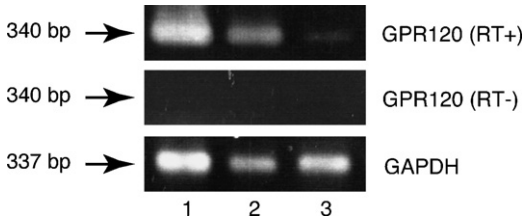

Fig. 1. Expression of GPR120 mRNA in the tongue, as determined by RT-PCR. GPR120 mRNA is expressed in the colon (positive control) (lane 1) and circumvallate papillae (lane 2), but not in the nonsensory lingual epithelium (lane 3).

1 h, the sections were incubated overnight with rabbit anti-GPR120 antibody (MBL, Nagoya, Japan) at room temperature. After several rinses in PBS, the sections were incubated with Alexa Fluor 546-conjugated donkey anti-rabbit IgG (Molecular Probes). After rinsing in PBS, the sections were incubated with rabbit anti-phospholipase-Cβ2 (PLCβ2; Santa Cruz, Santa Cruz, CA), anti-α-gustducin or anti-neuronal cell adhesion molecule (NCAM; Chemicon, Temecula, CA) antibodies labeled with Zenon-Alexa 488 (Molecular Probes) for 1 h. After washing with PBS, sections were refixed with 4% paraformaldehyde for 15 min and coverslipped using an antifading glycerol-based mounting medium. Immunostained sections were observed under a confocal laser scanning microscope (Olympus, Tokyo, Japan) with Ar and He–Ne laser sources.

Control staining was conducted without primary antibody or with preabsorbed antibody, i.e., with diluted antibody that had been

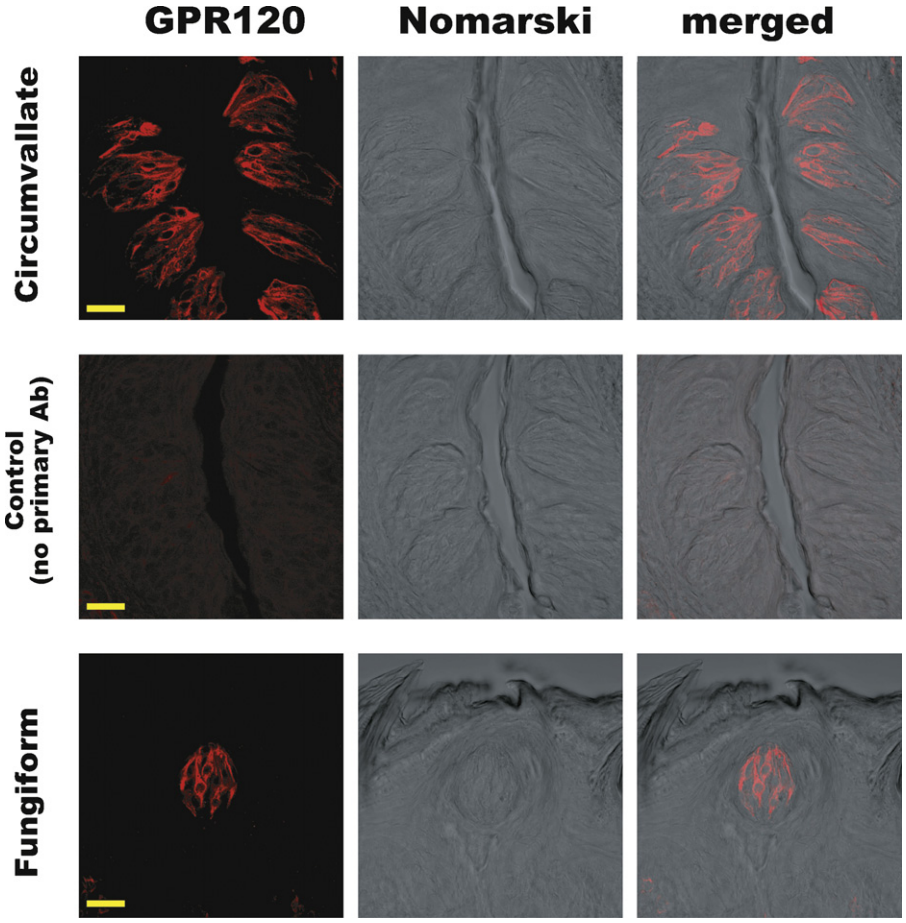

Fig. 2. Immunohistochemical staining for GPR120 in the lingual epithelium. GPR120 immunoreactivity was detected in the taste cells of the circumvallate papillae (top panel). No immunoreactivity was observed in the absence of primary antibody (control) (middle panel). GPR120 immunoreactivity was also observed in the fungiform papillae (bottom panel). Scale bars = 20 μm.

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