Expression of the putative membrane fatty acid transporter (FAT) in taste buds of the circumvallate papillae in rats

Tsutomu Fukuwatari[a], Teruo Kawada[b], Miho Tsuruta[b], Takenori Hiraoka[b], Toshihiko Iwanaga[c], Etsuro Sugimoto[a], Tohru Fushiki[b,*]

[a]*School of Human Culture, University of Shiga Prefecture, 2500 Hassaka-cho, Hikone, Shiga 522, Japan*
[b]*Laboratory of Nutrition Chemistry, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-01, Japan*
[c]*Laboratory of Anatomy, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Kita-ku, Sapporo 060, Japan*

Received 21 July 1997

Abstract The putative membrane fatty acid transporter (FAT) protein and its mRNA, originally expressed in adipose tissue, were found in the tongue of rats. Northern blot analysis showed a significant expression of FAT mRNA in the epithelial layer of circumvallate papillae. Immunohistochemical staining revealed that immunoreactivity for FAT is specifically localized in the apical part of taste bud cells, possibly gustatory cells, in the circumvallate papillae.

Key words: Fatty-acid transporter; Fatty-acid-binding protein; Lingual epithelium; Taste bud

1. Introduction

Fat in food is not only a source of essential nutrients, but also plays an important role in taste sensation. It is generally known that some laboratory animals, such as rats and mice, have a preference for high fat diets [1]. Much attention has been paid to the involvement of fat and triglycerides in food texture [2,3], while few studies have been made on the sensation of fat, mainly because the size of oil droplets has been considered too large to bind to sensory receptors [4]. However, Mattes [5] has shown that postprandial lipid metabolism is affected by exposure of the oral cavity to dietary fat, suggesting recognition of dietary fat in the oral cavity. Recently, Gilbertson et al. [6], using the patch-clamp recording method, showed that the delayed-rectifying K^+ channel of taste buds cells was inhibited by *cis*-unsaturated long chain fatty acids (LCFA), and suggested the presence of a sensory mechanism for fat in taste receptor cells.

In mammals, five kinds of proteins have been identified as LCFA-binding proteins present on the cell membrane. They are the 22 kDa 3T3-L1 adipocyte plasma membrane protein [7], 40–43 kDa plasma membrane fatty-acid-binding protein (FABPpm), which is identical to the isoform of mitochondrial aspartate aminotransferase (mAspAT) [8–10], 50–60 kDa protein purified from the kidney and heart [11], 63 kDa fatty-acid transport protein (FATP) [12], and 88 kDa fatty-acid transporter (FAT), which is 85% similar to human CD36 glycoprotein, and thus likely to be the rat homolog of CD36 [13,14]. Among them, FABPpm and FAT are expressed in the small intestine which possesses a variety of chemoreceptors for dietary components. Poirier et al. [15] showed that FAT in the jejunal mucosa is expressed in the brush border of epithelial cells, and FAT mRNA in the small intestine is increased by a LCFA-rich diet, suggesting the participation of FAT in the sensation or absorption of dietary fat. We considered that if FAT or related proteins are involved in the sensation of dietary fat in the oral cavity, they must be expressed in taste organ. In this study, we examined the expression and distribution of FAT mRNA in taste buds of circumvallate papillae in rats.

2. Materials and methods

2.1. Reverse transcription-polymerase chain reaction (RT-PCR)

According to the highly conserved region of the CD36 gene family [16], the three oligonucleotide primers, primer 1 (complement to 727–746) 5′-GT(CG)CCATT(AG)ATCATGT(CT)(AG)CA-3′, primer 2 (complement to 521–540) 5′CCACAAGAGTTCCTTCAAAC-3′ and primer 3 (complement to 263–283) 5′-CTCTGTATGTGTAAGGACCTC-3′ were prepared. The tongue and epididymal fat pad for control were dissected out from 20 male Wistar rats weighing about 180 g. The lingual epithelium was isolated from the tongue according to Strien et al. [17], and the epithelium of circumvallate papillae was cut off. RNA was extracted from the circumvallate papillae by the AGPC method [18], and poly(A^+) RNA was prepared from the total RNA using Oligotex-dT30 latex. The reverse transcript of poly(A^+) RNA was obtained using primer 1, and the poly(A) tail was added to the 3′ terminal of ss cDNA obtained. The PCR reaction was performed using 5′ poly(A)-tailed cDNA as template, and using primer 2 and the dT17-adapter primer, 5′-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3′. The secondary PCR reaction was performed using the first PCR product as template, and using primer 3 and adapter primer, 5′-GACTCGAGTCGACATCGA-3′. Conditions for PCR were: 94°C, 30 s, 55°C, 1 min, 72°C, 2 min, for 35 cycles. Gel analysis revealed that the amplified DNA fragments were about 600 bp. After subcloning with pUC18, the amplified DNA fragments were sequenced by the dideoxy method [19].

2.2. Northern blot analysis

The lingual epithelium and epididymal fat pad were used for Northern blot analysis. The whole epithelial layer isolated from the tongue was divided into three parts: (1) circumvallate papillae, (2) the epithelium covering the posterior one third of the tongue except for the circumvallate papillae (tongue nonsensory epithelium), and (3) the epithelium covering the anterior two thirds of the tongue (anterior tongue epithelium). RNA from these three types of epithelia was obtained by the AGPC method [18]. RNA after fractionation on agarose gel was transferred onto nylon membrane, and hybridized with randomly primed ^{32}P-labeled rat FAT cDNA probe or rat β-actin cDNA probe at 42°C. After washing, it was subjected to autoradiography at −70°C using Fuji AIF X-ray film.

*Corresponding author. Fax: (81) (75) 753-6264.
E-mail: d53765@sakura.kudpc.kyoto-u.ac.jp

Abbreviations: LCFA, long chain fatty acid(s); FAT, fatty-acid transporter; RT-PCR, reverse transcription-polymerase chain reaction

The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession number AB005743.

PII S0014-5793(97)01055-7

```
PFC                                CTTTG AAGAGATCAG TGACTCTGTA ACCTTGCAAC TGCATTTTGA TGGTTCCTTT  -241

hCD36                                    GAAAAATCCT TCTTAGCCAT TTTAAAGATA GCTTTCCAAT GATTAGACGA  -161
PFC   AAACTCGCGG ACTTGTACTC TCTCCTCGGA TGGCTAGCTG ATTACTTCTG TGTAGCAGCT TACAAATGAT TCGAAGTGTT

hCD36 ATTGATTCTT TCTGTGACTC ATCAGTTCCT TTCCTGTAAA ATTCATGTCT TGCTGTTGAT TTGTGAATAA GAACCAGAGC   -81
PFC   GAAACTTTCT GAGTCTCAAT GAACTATTTC TCACACAACT CAGATACTGC TGTTCATGCA TGAATTAGTT GAACCAGGCC

hCD36 TTGTAGAAAC CACTTTAATC ATATCCAGGA GTTTGCAAGA AACAGGTGCT TAACACTAAT TCACCTCCTG AACAAGAAAA    -1
FAT          CGG CATTGTAATT GTACCTGTGA GTTGGCAAGA AGCAAGTGCT CTTCCTTGAT TCTGCTGCAC GAGGAGGAGA
PFC   ACATAGAAAG CATTGTAATT GTACCTGTGA GTTGGCAAGA AGCAAGTGCT CTTCCTTGAT TCTGCTGCAC GAGGAGGAGA

hCD36 ATGGGCTGTG ACCGGAACTG TGGGCTCATC GCTGGGGCTG TCATTGGTGC TGTCCTGGCT GTGTTTGGAG GTATTCTAAT   80
FAT   ATGGGCTGCG ATCGGAACTG TGGGCTCATT ACTGGAGCCG TTATTGGTGC TGTCCTGGCT GTGTTTGGAG GCATTCTCAT
PFC   ATGGGCTGCG ATCGGAACTG TGGGCTCATT ACTGGAGCCG TTATTGGTGC TGTCCTGGCT GTGTTTGGAG GCATTCTCAT

hCD36 GCCAGTTGGA GACCTGCTTA TCCAGAAGAC AATTAAAAAG CAAGTTGTCC TCGAAGAAGG TACAATTGCT TTTAAAAATT  160
FAT   GCCGGTTGGA GACCTACTCA TTGAGAAGAC AATCAAAAGG GAAGTTGTCC TTGAAGAAGG AACCATTGCT TTCAAAAACT
PFC   GCCGGTTGGA GACCTACTCA TTGAGAAGAC AATCAAAAGG GAAGTTGTCC TTGAAGAAGG AACCATTGCT TTCAAAAACT

hCD36 GGGTTAAAAC AGGCACAGAA GTTTACAGAC AGTTTTGGAT CTTTGATGTG CAAAATCCAC AGGAAGTGAT GATGAACAGC  240
FAT   GGGTGAAAAC GGGCACCACT GTGTACAGAC AGTTTTGGGT CTTTGACGTG CAAAACCCAG AGGAAGTGGC AAAGAATAGC
PFC   GGGTGAAAAC GGGCACCACT GTGTACAGAC AGTTTTGGAT CTTTGACGTG CAAAACCCAG AGGAAGTGGC AAAGAATAGC

hCD36 AGCAACATTC AAGTTAAGCA AAGAGGTCCT TATACGTACA GAGTTCGTTT TCTAGCCAAG                        300
FAT   AGCAAGATCA AGGTTATACA GAGAGGTCCT TACACATACA GAGTTCGCTA TTTAGCCAAG
PFC   AGCAAGATCA AGGTTAAACA GAGAGGTCCT TACACATACA GAG
```

Fig. 1. Alignment of partial nucleotide sequences of human CD36, rat FAT, and RT-PCR clone encoding putative FAT in circumvallate papillae (PFC). Nucleotides are numbered in the 5′ to 3′ direction from the first nucleotide of the putative initiation codon. Primers used for RT-PCR are underlined.

2.3. Immunohistochemical staining

Another five rats were perfused via the aorta with physiological saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3. Lingual tissues containing the circumvallate papillae were dissected out and immersed in the same fixative for an additional 6 h. After dipping in 30% sucrose overnight, the tissues were quickly frozen in liquid nitrogen. Cryostat sections, about 15 μm in thickness, were prepared and processed with the avidin-biotin complex (ABC) method using the anti-FAT serum. The sections were incubated with the antiserum diluted in 1:3000 overnight. The antigen-antibody reaction was detected using a streptavidin-biotin kit (Histfine, Nichirei, Tokyo).

3. Results and discussion

To examine whether FAT or related proteins are expressed in circumvallate papillae, oligonucleotide primers were prepared according to a highly conserved region of the CD36 gene family [16] in which FAT is included, and RT-PCR reaction was performed with the 5′ RACE method [20] using poly(A^+) RNA obtained from the rat circumvallate papillae. The RT-PCR product of circumvallate papillae was about 600 bp, similar in size to that of rat adipose tissue. After subcloning of the RT-PCR product from circumvallate papillae, the nucleotide sequence of the positive clone was determined, and the RT-PCR clone was found to be same as FAT (Fig. 1).

To obtain further information on the expression of FAT gene on lingual epithelium, Northern blot analysis using FAT cDNA was performed for RNAs obtained from circumvallate papillae, surrounding nonsensory epithelium which lacks taste buds (tongue nonsensory epithelium), and anterior tongue epithelium, where fungiform papillae with a few taste buds are scattered. Adipose tissue RNA from the epididymis was also used as an internal control. In circumvallate papillae, a small but significant amount of FAT mRNA was expressed (Fig. 2, lane 3). However, no FAT mRNA was detected in the tongue nonsensory epithelium and the anterior tongue epithelium (Fig. 2, lanes 1, 2).

Since FAT mRNA was found to be expressed specifically in the epithelium of circumvallate papillae, immunohistochemical analysis of the tongue was performed using anti-FAT antibody to identify the expression site of FAT in circumvallate papillae [21]. Immunoreactivity for FAT was localized exclusively in taste buds, in which immunoreactive cells and immunonegative cells were intermingled (Fig. 3). In immunoreactive taste bud cells, the apical part of cells was intensely labeled (Fig. 3). Western blot analysis using the same anti-FAT serum failed to detect the band corresponding to FAT in circumvallate papillae as well as in the tongue nonsensory epithelium and the anterior tongue epithelium (data not shown), indicating that a very small amount of FAT protein may be expressed in circumvallate papillae.

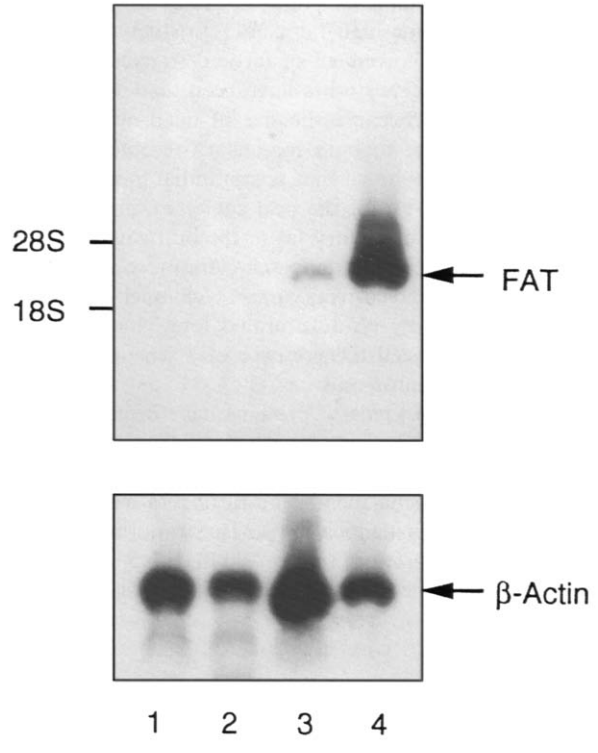

Fig. 2. Northern blot analysis of FAT in tongue epithelium and adipose tissue of rat. Electrophoresis was obtained using 7 μg total RNA obtained from lingual epithelium separated into anterior tongue fungiform (lane 1), tongue nonsensory epithelium (lane 2), and circumvallate papillae (lane 3). Lane 4 shows RNA from adipose tissue.

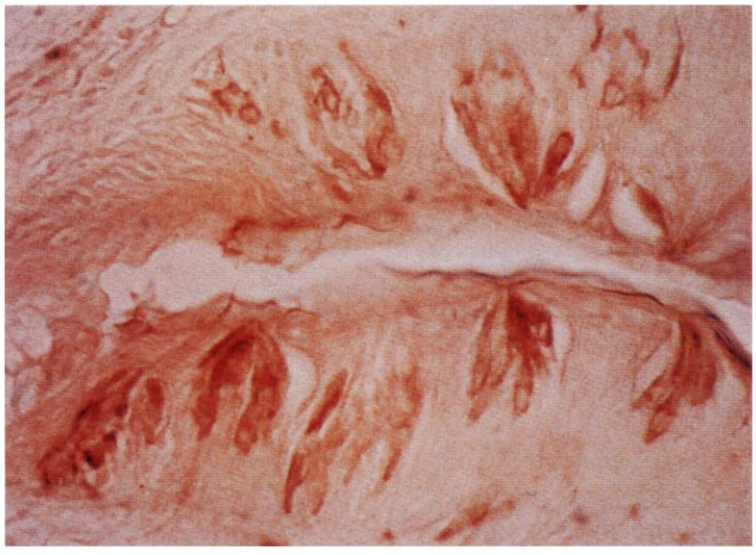

Fig. 3. Immunohistochemical staining of rat circumvallate papillae with anti-rat FAT. Some cells in each taste bud are stained positively with more intense labeling in the apical part of cells.

In the rat tongue, mRNA of FAT was specifically expressed in the epithelium of circumvallate papillae, and the immunoreactivity for FAT was restricted to the taste buds, mainly in the apical part of the taste bud cells. These findings strongly suggest the involvement of FAT in taste sensation of the circumvallate papillae. FAT was isolated and purified from adipocytes as a protein combined with sulfo-*N*-succinimidyl which is a derivative of oleic acid [13]. FAT increases the uptake of fatty acid when expressed in the Ob17PY fibroblast, and combines with LCFA reversibly [22], although its function remains unknown. FAT and CD36 on cell membrane are considered to have a major extracellular moiety and one or two transmembrane regions in each molecule [14,23,24]. Since FAT and CD36 differ in structure from most of the membrane carriers with many transmembrane regions, they are considered to function as receptors for LCFA rather than LCFA transporters. The fact that FAT has a region which interacts with Src kinase at the C-terminal suggests a role in the signal-transferring process initiated by binding with LCFA [14,25,26]. This idea is partially supported by the predominant localization of FAT immunoreactivity in the apical region of taste bud cells. Therefore, FAT may play a role in cellular signal transmission by binding diet-derived LCFA at the apical cell surface of taste buds rather than responding at the basolateral cell surface.

For detection of fat by taste buds, fat must be digested into LCFA in the oral cavity. The circumvallate papillae are associated with the proper salivary glands called Ebner's glands. The excretory ducts of the glands are open to the bottom of the circular furrow. All taste buds lined along the furrow are exposed directly to the fluid secreted from Ebner's gland. Several studies revealed a rich existence of lipases in the secretions from Ebner's gland [27–29]. Therefore, LCFA generated from triglyceride may be carried to the taste cells by lipophilic carrier molecules, and sensed as the presence of fat in the diet.

The intake of food is known to be rapidly regulated through chemoperception of diet-derived molecules before absorption in the small intestine [30]. α-Gustducin, which is the α-subunit of trimeric GTP-binding protein complex expressed in taste buds [31], is also found in the intestinal epithelium [32]. Since α-gustducin participates in intracellular signal transfer of bitterness and sweetness by acting with the taste receptors on cell membrane [33], the chemoperception mechanism for the diet-derived molecules in taste cells is considered similar to that in the digestive tract. Previously, we suggested the participation of receptor protein in the fat-sensory mechanism of intestinal epithelial cells [34,35]. In addition, selective expression of FAT in the brush border of jejunal epithelial cells suggests that FAT participates in sensation or absorption of dietary fat [15]. Thus, we conclude that some dietary fat detecting system similar to that in the intestinal epithelium exists in taste cells, and that FAT participates in the sensory mechanism. These findings provide clues to the long-sought molecular and cellular basis for chemoreception in the oral cavity.

Acknowledgements: We are indebted to Drs. Nada A. Abumrad and Dr. Azeddine Ibrahimi (Department of Physiology and Biophysics, State University of New York) for the generous gift of the FAT antibody.

References

[1] Hamilton, C.L. (1964) J. Comp. Physiol. Psychol. 58, 459–460.
[2] Elizalde, G. and Sclafani, A. (1990) Appetite 15, 189–197.
[3] Ramirez, I. (1992) Appetite 18, 193–206.
[4] Ramirez, I. (1991) Physiol. Behav. 50, 389–392.
[5] Mattes, R.D. (1996) Am. J. Clin. Nutr. 63, 911–917.
[6] Gilbertson, T.A., Fontenot, D.T., Liu, L., Zhang, H. and Monroe, W.T. (1997) Am. J. Physiol. 272, C1203–C1210.
[7] Trigatti, B.L., Mangroo, D. and Gerber, G.E. (1991) J. Biol. Chem. 266, 22621–22625.
[8] Stremmel, W., Strohmeyer, G., Borchard, F., Kochwa, S. and Berk, P.D. (1985) Proc. Natl. Acad. Sci. USA 82, 4–8.
[9] Berk, P.D., Wada, H., Horio, Y., Potter, B.J., Sorrentino, D., Zhou, S.-L., Isola, L.M., Stump, D., Kiang, C.-L. and Thung, S. (1990) Proc. Natl. Acad. Sci. USA 87, 3484–3488.
[10] Stump, D.D., Zhou, S.-L. and Berk, P.D. (1993) Am. J. Physiol. 265, G894–G902.
[11] Fujii, S., Kawaguchi, H. and Yasuda, H. (1987) Lipids 22, 544–546.
[12] Schaffer, J.E. and Lodish, H.F. (1994) Cell 79, 427–436.
[13] Harmon, C.M., Luce, P., Beth, A.H. and Abumrad, N.A. (1991) J. Membr. Biol. 121, 261–268.

[14] Abumrad, N.A., el-Maghrabi, M.R., Amri, E.Z., Lopez, E. and Grimaldi, P.A. (1993) J. Biol. Chem. 268, 17665–17668.
[15] Poirier, H., Degrace, P., Niot, I., Bernard, A. and Besnard, P. (1996) Eur. J. Biochem. 238, 368–373.
[16] Striem, B.J., Naim, M. and Lindemann, B. (1991) Cell Physiol. Biochem. 1, 46–54.
[17] Vega, M.A., Segui-Real, B., Garcia, J.A., Cales, C., Rodriguez, F., Vanderkerckhove, J. and Sandoval, I.V. (1991) J. Biol. Chem. 266, 16818–16824.
[18] Chomczynski, P. and Sacci, N. (1987) Anal. Biochem. 162, 156–159.
[19] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[20] Fohman, M.A. (1990) in: M.A. Inssi, D.H. Gelfand, J.J. Sninsky and T.J. White (Eds.), PCR Protocols. Academic Press, London, pp. 28–38.
[21] Ibrahimi, A., Sfeir, Z., Magharaie, H., Amri, E.-Z., Grimaldi, P. and Abumrad, N.A. (1996) Proc. Natl. Acad. Sci. USA 93, 2646–2651.
[22] Baillie, A.G.S., Coburn, C.T. and Abumrad, N.A. (1996) J. Membr. Biol. 153, 75–81.
[23] Greenwalt, D.E., Lipsky, R.H., Ockenhouse, C.F., Ikeda, H., Tandon, N.N. and Jamieson, G.A. (1992) Blood 80, 1105–1115.
[24] Frieda, S., Pearce, A., Wu, J. and Silverstein, R. (1994) Blood 84, 384–389.
[25] Huang, M.M., Bolen, J.B., Barnell, J.W., Shattil, S.J. and Brugge, J.S. (1991) Proc. Natl. Acad. Sci. USA 88, 7844–7848.
[26] Schuepp, B.J., Pfister, H., Clemetson, K.J., Silverstein, R.L. and Jungi, T.W. (1991) Biochem. Biophys. Res. Commun. 175, 263–270.
[27] Field, R.B., Spielman, A.I. and Hand, A.R. (1989) J. Dent. Res. 68, 139–145.
[28] Hamosh, M. and Hand, A.R. (1977) Lab. Invest. 37, 603–608.
[29] Schmale, H., Holtgreve-Grez, H. and Christiansen, H. (1990) Nature 343, 366–369.
[30] Mei, N. (1985) Physiol. Rev. 65, 211–237.
[31] McLaughlin, S.K., McKinnon, P.J. and Margolskee, R.F. (1992) Nature 357, 563–569.
[32] Hofer, D., Puschel, B. and Drenckhahn, D. (1996) Proc. Natl. Acad. Sci. USA 93, 6631–6634.
[33] Wong, G.T., Gannon, K.S. and Margolskee, R.F. (1996) Nature 381, 737–738.
[34] Shintani, T., Takahashi, N., Fushiki, T. and Sugimoto, E. (1995) Biosci. Biotech. Biochem. 59, 479–481.
[35] Shintani, T., Takahashi, N., Fushiki, T. and Sugimoto, E. (1995) Biosci. Biotech. Biochem. 59, 1428–1432.