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Legends of Supplementary Figures

Figure 1: Effects of LA and GA on Ca^{2+} signaling in human fungiform TBC. The cultured TBC ($2x10^5$/assay) were loaded with Fura-2/AM and the changes in intracellular Ca^{2+} (F_{340}/F_{380}) monitored. **A** and **B**: Increase in iCa^{2+} when LA or GA were added at 20 µM individually or combined. **C**: LA or GA added at 100 µM. Data in B and C are means ± SEM (n=7). NS= non-significantly different.

Figure 2: LA and GA induce IP_3 production in human fungiform TBC. The cultured TBC were exposed to 20 µM LA or GA for 20 min and IP_3 production was determined. The data are means ± SEM (n=5). NS= non-significantly different.

Figure 3: CD36 and GPR120 expression in control STC-1 (Ctrl) and in STC-1 stably expressing human CD36 (+CD36). A: Western blots showing CD36 and GPR120 proteins with actin as the loading control. **B:** Effects of SSO on Ca^{2+} signaling in CD36 positive STC-1 cells. Cells ($2x10^5$/assay) were pretreated with SSO (20 µM, 15 min) before addition of LA and $[Ca^{2+}]i$ imaging. The data shown are representative of n=3 experiments

Figure 4: High fat feeding reduces LA-induced serotonin release and enhances GA-induced GLP-1 secretion by mice fungiform TBC. A: Release of ^3H-5-HT by TBC from mice fed a standard diet (Std) or a high fat diet (HFD) for two months (n=7). Serotonin levels were determined as described in supplementary Materials and Methods. **B:** Effects of LA and GA on TBC release of GLP-1 (determined as described in Supplementary Materials and Methods). NS=non-significantly different. Values are means ± SEM (n=4).

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Supplementary materials and methods

Materials

All other chemicals including MCDB153 medium were from Sigma (USA). Grifolic acid (GA) was purified from *Albatrellus dispansus.*[1] The antibodies for CD36 were from R&D (UK) (AF 2519) and Santacruz (USA) (sc-52645), those for GPR120 from Santacruz (USA) (sc-48203), Molecular Biology Lab (USA) (LS-A2003) and Abcam (ab75313), for α-gustducin (sc-395) and PLC-β2 (sc-206) Santacruz (USA) and for caveolin 1 BD Biosciences (Le Pont de Claix, France).

Cultures and immunocytochemistry of human fungiform TBC

The CD36-positive TBC, by using a positive selection technique, were isolated as described for mice TBC.[2] The TBC were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2) for 1 hour at room temperature. For immunocytochemistry, primary antibodies were used at 1:500 (anti-α-gustducin, anti-PLC-β2), 1:250 (GPR120) or 1:50 (CD36) and secondary antibodies were anti-rabbit IgG Alexa 635 or anti-mouse IgG Alexa 488 (Molecular Probes, USA). Images were acquired (Leica TCS SP2 Spectral Confocal Microscope) at 405 nm for DAPI, 488 nm for Alexa Fluor 488, and 633nm for Alexa Fluor 633.

Culture of mouse fungiform TBC

The CD36-positive TBC, by using a positive selection technique, from mouse lingual fungiform papillae were isolated as described elsewhere.[2] The TBC were seeded onto a Biocoat Poly-D-lysine coated dishes and cultured for 24 h before being used for experiments.

Culture of STC-1 cells

The STC-1 cells were cultured in DMEM medium with 20% FBS. STC-1 cells expressing human *CD36* were generated by electroporation (Nucleofector Kit V, Lonza, Germany) as previously described.[3]

Measurement of IP_3 production

The human fungiform TBC cells ($1x10^6$) were treated with LA (20 µM) or GA (20 µM) for 20 min. Combined treatment was carried out by adding consecutively LA and GA (or vice versa) in 10 min intervals. The controls received 0.1 % ethanol (v/v) for 20 min. After stimulation, the cells were washed twice with ice-cold PBS and lysed with 20% ice-cold perchloric acid (0.2:1; v/v). After centrifugation, the supernatant was assayed for IP_3 production using a kit (NEK064) from Perkin Elmer (France) according to the manufacturer's instructions.

mRNA detection by Real Time-quantitative PCR (RT-qPCR)

Total RNA, extracted from TBC by Trizol (Invitrogen), treated with DNAse (RNAse-free,Qiagen) and reverse transcribed (1µg) with Super script II RNAse H-reverse transcriptase (Invitrogen). RT-qPCR was performed with the iCycler iQ real time detection system by employing SYBR Green I. Primers used (forward; reverse) were: β-actin: 5'-AGAGGGAAATCGTGCGTGA-3'; 5'-CAATAGTGATGACCTGGCCGT-3', GPR120: 5'-CTGGGGCTCATCTTTGTCGT-3'; 5'-ACGACGAGCACTAGAGGGAT-3', CD36: 5'-GGCCAAGCTATTGCGACATG-3'; 5'-CCGAACACAGCGTAGATAGAC-3', α-Gustducin: 5'-ACACATTGCAGTCCATCCTAGC-3'; 5'-ATCACCATCTTCTAGTGTATTTGCC-3'.

siRNA knock-down of CD36 and GPR120

Human fungiform TBC and STC-1 cells were transfected with siRNA ON-Target plus Smart pool (25 nM) or non-targeting siRNA as a control (Dharmacon) using the DharmaFECT protocol (Dharmacon). Target sequences for CD36 siRNA and GPR120 siRNA were, respectively: 5'-GGAAAGUCACUGCGACAUG-3' and 5'-GGAAGAGGCUCACGGUAAG-3'. Twenty-four hours before siRNA treatment, the cells were placed in the culture medium without antibiotics. After transfection (24-h), the medium was replaced with fresh medium without siRNA for 48 h before performing experiments.

Lipid Raft Isolation

Lipid rafts were isolated by sucrose density gradient centrifugation.[4] Briefly, control and LA-treated (20 minutes) fungiform TBC ($2.5x10^7$) were washed with PBS, lysed on ice in 2 ml of 1% Triton X-100 in 25 mM morpholineethanesulfonic acid, 150 mM NaCl, pH 6.5, buffer with a protease inhibitor cocktail, and homogenized (Dounce homogenizer). Homogenates equalized based on protein concentrations, mixed with 2 ml of 80% sucrose in 1% Triton X-100, 25 mM morpholineethanesulfonic acid, 150 mM NaCl, pH 6.5, buffer, were placed in a centrifuge tube, overlaid with 4 ml of 30% sucrose and 4 ml of 5% sucrose, and centrifuged at 175,000xg for 20 hours at 4°C (SW41 rotor, Beckman Ultracentrifuge). Ten fractions (1.2 ml each) were collected from the top of the gradient and fractions 3–6 were precipitated with 2 volumes of cold acetone for immunoblotting. Caveolin was the positive control for lipid raft detection and β-actin was used for soluble fractions.

Western blots

The TBC were lysed in 50 µl buffer (20 mM HEPES, pH 7.3; 1 mM EDTA; 1 mM EGTA; 0.15 mM NaCl; 1% Triton X-100; 10% glycerol; 1 mM phenylmethylsulfonyl fluoride; 2 mM sodium orthovanadate and 2 µl/ml anti-protease cocktail) and centrifuged

(13000*g* x 10 min). Denatured proteins (30 µg) were separated by SDS-PAGE (10%), transferred to polyvinylidine difluoride membranes, and probed for CD36 (anti-CD36 antibody, 1:500 dilution) and GPR120 (anti-GPR120, 1:500 dilution). Signal quantification was done by ChemiDoc XRS System (Bio-Rad).

High-fat diet

The standard diet contained 4% fat[5] and had the following FA composition (% of total FA): C14:0 (0.73), C16:0 (6.60); 18:0 (2.36); C18:1 (60.09); C18:2 n-6 (19.41) and C18:3 n-3 (8.88). The HFD was the same as the standard diet except it contained 40% fat (palm oil) with the following FA composition (% of total FA): C14:0 (1.25), C16:0 (43.49); 18:0 (4.46); C18:1 (40.32); C18:2 n-6 (9.93) and C18:3 n-3 (0). Diets were prepared every day and the mice consumed them *ad libitum.*

Two-bottle preference test of lipid solution

The experiments on the spontaneous preference for lipid-enriched solutions were performed by two-bottle preference test.[6] The individually caged mice, fed either a standard diet or a HFD, were allowed to choose between 0.1% canola oil emulsified in 0.3% xanthan gum (w/v) in water or water with 0.3% xanthan gum (w/v) over a period of 12h. The canola oil contained as % of fatty acids: 61%, oleic acid (18:1 n-9), 25 %, linoleic acid (18:2 n-6) and 10 %, rumelenic acid (18:3 n-3).[7] The intake was determined by weighing the feeders.

Release of [^{3}H]serotonin

The release of [^{3}H]serotonin was determined according to our previously published procedure.[6] Chlorgyline (100 µM) and pargyline (100 µM) were used in order to prevent serotonin metabolism. [^{3}H] serotonin release was calculated as percent of unstimulated cells.

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Detection of GLP-1 release

Human fungiform TBC or freshly isolated TBC from standard diet or HFD fed mice were cultured in the presence of LA (20 µM) or GA (20 µM) for 2 h. The GLP-1 concentrations in the supernatants were determined by ELISA (Millipore) according to the protocol furnished with the kit and mentioned elsewhere.[8]

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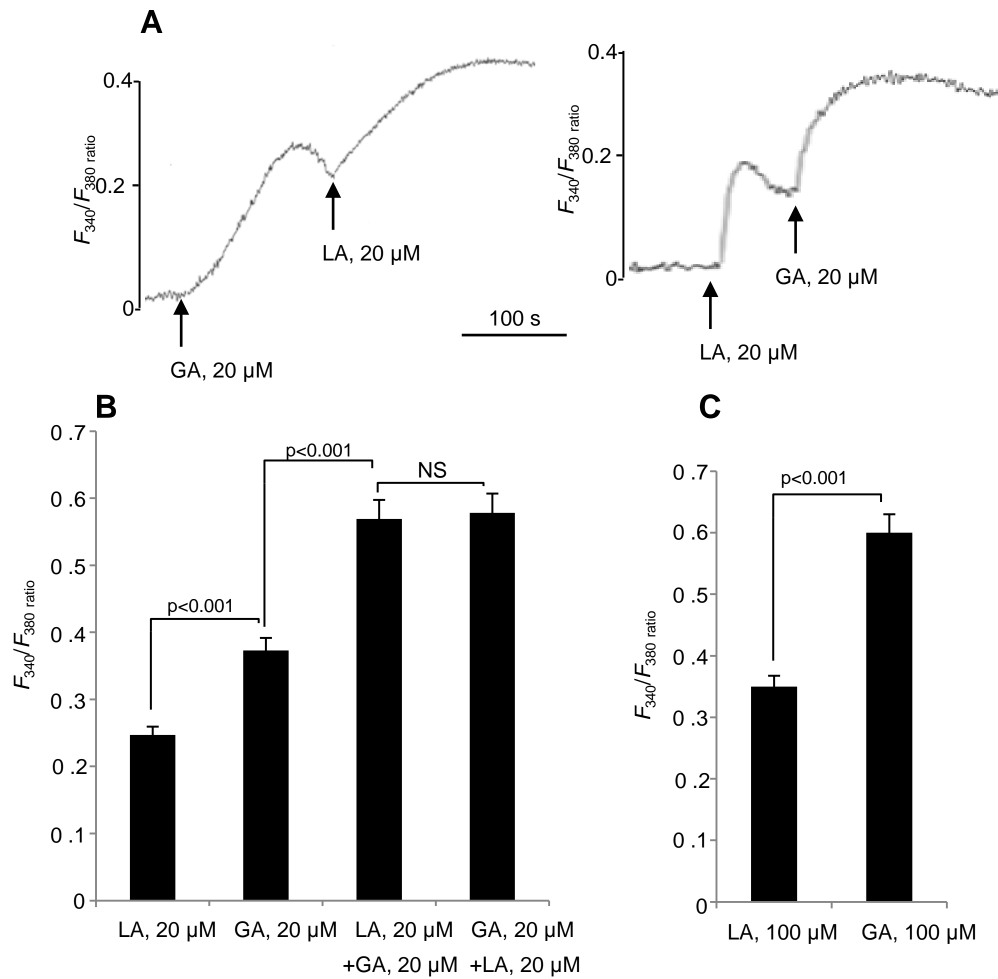
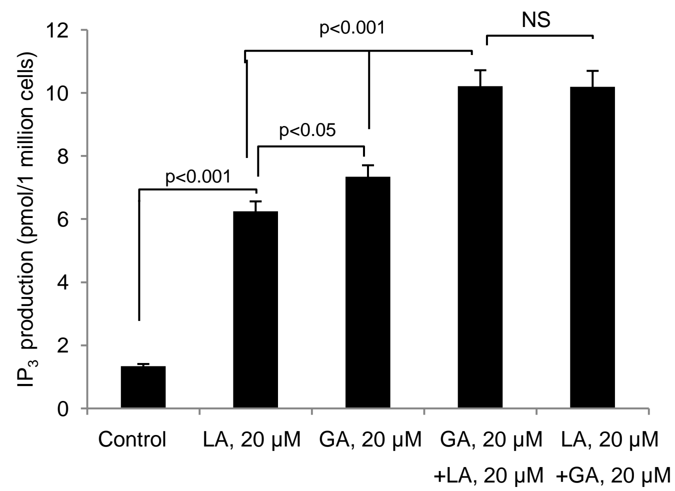
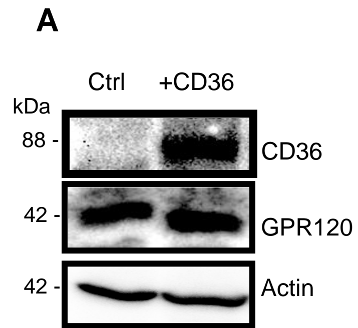
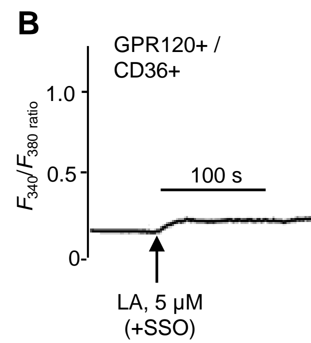


Supplementary Fig. 1

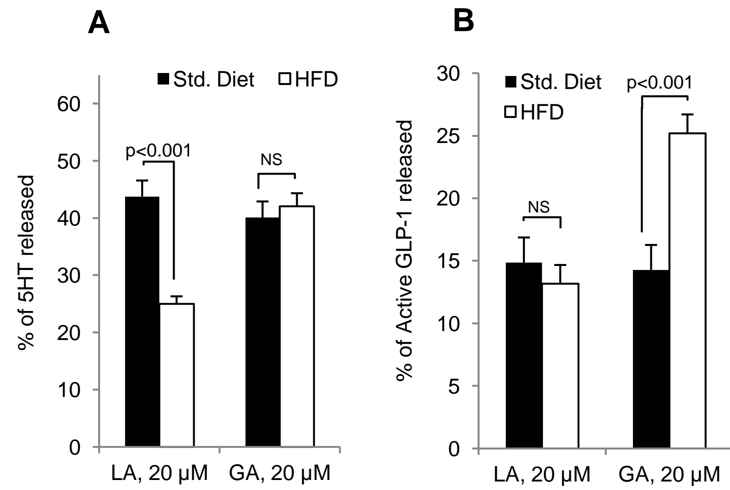


Supplementary Fig. 2





Supplementary Fig. 3



Supplementary Fig. 4