Fatty acid responses in taste cells from obesity-prone and -resistant rats

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Abstract

One of the transduction mechanisms for the chemoreception of fat has been proposed to involve the inhibition of delayed rectifying potassium (DRK) channels by polyunsaturated free fatty acids (PUFAs). In the present study we have compared the responsiveness of fungiform taste receptor cells (TRCs) to fatty acids in obesity-prone (Osborne–Mendel; O–M) and obesity-resistant (SSB/PI) rat strains using patch clamp recording. TRCs from SSB/PI rats were markedly more responsive to PUFAs than those from O–M, yet with identical inhibition constants. Moreover, addition of PUFAs to subthreshold concentrations of saccharin enhanced preference for the mixture in two-bottle preference tests compared to the saccharin alone in SSB/PI but not O–M rats. The correlation between electrophysiological and behavioral effects of PUFAs suggested that differences in fatty acid-sensitive DRK expression may underlie the phenotypic differences between SSB/PI and O–M rats. We propose that the ratio of fatty acid-sensitive DRK channels to fatty acid-insensitive DRK channels may be important to contributing to overall peripheral fatty acid sensitivity and in that way influence the strength of the resulting chemosensory response to fat.

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1. Introduction

The primary role of the taste system lies in its ability to recognize both nutrients needed for survival and compounds that potentially could lead to harm if ingested. With respect to the former, the taste system has mechanisms that enable it to recognize minerals, carbohydrates and proteins that correspond with the human perceptions of salty, sweet and umami, respectively. Fat, as well, is a necessary requirement in the diet, yet until recently it has been assumed that fat, and the components contained therein, did not directly affect taste receptor cells, but rather only were perceived by their textural properties [1,2] primarily detected through trigeminal stimulation [3,4]. Although the mechanism underlying textural perception has not been unequivocally elucidated, it is clear that fat contains textural cues, some of which humans refer to as “mouthfeel” and “oiliness”. In recent years, there have been a number of studies that have emerged which demonstrate the ability of components in fats, specifically free fatty acids, to activate taste receptor cells, consistent with there being a “taste of fat” ([5]; for review, see [6]).

The transduction of taste stimuli involves an interaction between the transient and ion channels or receptors localized in most cases on the apical membranes of the taste receptor cells (TRCs). Although the pathways between TRC ion channel/receptor activation and neurotransmitter release have not been completely worked out, the general consensus is that this interaction leads to depolarization (receptor potential), activation of voltage-dependent Na⁺ and K⁺ channels (action potentials), a rise in intracellular Ca²⁺ via influx through voltage-gated Ca²⁺ channels and/or release from intracellular stores, and finally transmitter release (for review, see [7–9]). To date the mechanisms that have been identified for the initial events in the taste transduction of free fatty acids include an inhibition of delayed rectifying potassium (DRK) channels [10,11] and the intracellular transport of free fatty acids by the fatty acid transporter, CD-36 [12]. Concomitant with a role for
the taste system in nutrient recognition, the specificity of DRK channels in the anterior tongue (fungiform taste buds) is limited to the essential (cis-polyunsaturated) fatty acids [10]. The posterior tongue, however, appears to be less specific, and taste cells in the foliate and circumvallate taste buds also responded to the monounsaturated fatty acids, palmitoleic and oleic acid, in a preliminary study [13].

The rapid rise in obesity in the Western world has been attributed to a variety of factors including a decrease in energy expenditure and an increase in macronutrient intake. In particular, there appears to be a strong correlation between total fat intake and obesity, particularly in the United States [14,15]. As such, an understanding of the mechanisms that the body uses to recognize fats, their role in fat intake and their developmental and hormonal plasticity would seem crucial to develop potential physiological and pharmacological treatments for diet-induced obesity. In the present study we have followed up in greater detail the role of DRK channels in fat chemoreception in two strains of rats (cf. [11]). One strain, the Osborne–Mendel (O–M) may be broadly classified as an obesity-prone, fat-prefering rat while the other, S5B/Pl (S5B), is obesity-resistant and carbohydrate preferring [16]. The two strains were compared for their electrophysiological responses in fungiform TRCs to a variety of fatty acids using patch clamp recording, for the ability of fatty acids to alter taste preference in behavioral assays, and for quantitative expression of DRK channels using quantitative real-time polymerase chain reaction (qPCR). Taken together, the results support the proposal that differences in DRK expression may contribute to the phenotypic differences between O–M and S5B rats and that these channels may play roles in helping to shape dietary preference and fat intake. Part of these results have appeared in abstract form [13].

2. Methods

All experiments were performed on adult (2–4 month) male Osborne–Mendel (O–M) and S5B/Pl rats that were maintained on a 12 h:12 h day/night cycle with normal rat chow (Purina #5001) and water provided ad libitum. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Utah State University and were performed in accordance with American Veterinary Medical Association guidelines. Unless indicated otherwise, all chemicals listed below were obtained from Sigma Chemical Corp., St. Louis MO.

2.1. Patch clamp recording

Details of fungiform TRC isolation have been described in detail previously [17]. Briefly, rats were euthanized by exposure to CO₂ in a closed chamber followed by cervical dislocation and their tongues were removed and placed in ice cold saline. Tongues were injected beneath the lingual epithelium with approximately 0.8–1.0 ml of normal physiological saline (Tyrodes; [18]) to which was added 2.5 mg/ml dispase II, 1 mg/ml collagenase A (Roche, Indianapolis, IN) and 1 mg/ml trypsin inhibitor (soybean). The tongue was placed in a Ca²⁺–Mg²⁺ free Tyrodes that contained 2 mM BAPTA (Molecular Probes), bubbled with O₂ and incubated for approximately 25–30 min at room temperature. Following incubation, the lingual epithelium was removed from the underlying muscle and pinned out in a dish containing Ca²⁺– Mg²⁺ free Tyrodes. Individual taste buds were moved individually by gentle suction from a fire-polished pipette (~150 µm bore) and plated immediately into a recording chamber containing Tyrodes.

Recordings were made from individual TRCs or TRCs maintained in the taste bud in the whole-cell variation of the patch clamp technique, and cells were selected based upon our established criteria [19]. Intracellular (pipette) solution contained (in mM): 140 KCl, 1 CaCl₂, 2 MgCl₂, 10 HEPES, 11 ethylene glycol-bis-(aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 3 ATP. The pH was adjusted to 7.2 with KOH. Borosilicate pipettes were pulled on a Sutter P-97 puller (Sutter Instruments, Novato CA) and subsequently fire-polished on a microforge (model MF-9; Narishige, East Meadow NY) to a resistance of 5–10 MΩ. Series resistance and cell capacitance were compensated optimally before the recording. For activation of delayed rectifying K⁺ currents, the voltage was usually stepped from a holding potential of ~80 mV (or in some experiments ~130 mV; see Fig. 5) to +40 mV in 10-mV increments. Commands were delivered and data were recorded with pCLAMP software (versions 8–9) interfaced to an Axopatch 200 B amplifier with a Digidata 1322 A/D board (Axon Instruments, Union City, CA). Data were collected at 10 kHz and filtered on-line at 2 kHz.

All fatty acids were made as stock solutions (15–25 mg/ml) in EtOH, evacuated with N₂ and stored at ~80 °C for up to 2 weeks until they were diluted for use immediately prior to the experiment. Fatty acids were diluted in Tyrodes containing 0.5 mM tetrodotoxin to inhibit voltage-activated Na⁺ currents (and facilitate K⁺ current analysis) and applied by bath perfusion. Following fatty acid application TRCs were washed with Tyrodes containing 1 mg/ml fatty acid-free BSA for several minutes to promote rapid recovery of pretreatment DRK currents. For analysis, currents during fatty acid application were averaged over a consistent time range corresponding to the steady-state condition and compared with currents in control (Tyrode) solution. Tyrode solution was perfused between each application of fatty acids and continued until currents returned near pretreatment levels, which took several minutes with fatty acid-free BSA-containing Tyrodes. For analysis of delayed-rectifying K⁺ channels, currents were measured at a command potential of +40 mV during steady state. Significant effects of these compounds on K⁺ currents were determined by paired Student’s t-tests (α=0.05) compared with control currents immediately preceding the test stimulus. For generation of concentration–response curves, some TRCs were treated with fatty acids in an ascending concentration series, while others were tested in random order with interspersed BSA washes. No differences were seen using these two methods. Data are presented as mean ± SD, unless otherwise indicated.
2.2. Preference tests

A series of 48-h, two-bottle preference tests was performed to evaluate the ability of a polyunsaturated fatty acid (linoleic acid) and a saturated fatty acid (lauric acid) to alter preference for a subthreshold concentration of saccharin (0.5 mM). Four groups of 8 rats each (2 groups of O−M and 2 groups of S5B) were tested in this paradigm. Briefly, each group underwent 5 separate preference tests, each versus water: (1) saccharin (0.5 mM), (2) 5 µM fatty acid, (3) 20 µM fatty acid, (4) saccharin + fatty acid (5 µM) and (5) saccharin + fatty acid (20 µM). The four groups were tested with these solutions in random order. The concentrations of fatty acids chosen represented ~0.5× and ~2.0× the highest concentration of PUFAs tested on DRK channels in our electrophysiological experiments (see Table 2).

After each 24-h period, fluid intake was measured, bottles were replaced with fresh solutions, and the side (left versus right) of the test solution was altered to compensate for any innate side preference. Preference ratios were calculated as the amount of test solution intake over the same period. Thus, a preference ratio of 0.5 indicates the test solution was neither preferred nor avoided relative to water. Within each of the four groups, data were analyzed by one-way ANOVA followed by Bonferroni’s post hoc analysis and significance was set at α = 0.05.

2.3. Quantitative real-time PCR

Taste buds were isolated from the fungiform papillae of the O−M and S5B tongues as described above. Taste buds were washed individually several times to remove non-adherent cells and immediately placed into 1.5 ml microfuge tubes with 200 µl RNAlater (Ambion, Austin, TX) on ice. The taste buds were centrifuged at 6000 rpm (3300 g) for 7 min. The resulting pellet was resuspended in lysis buffer from the RNeasy Mini Kit (Qiagen, Valencia, CA), mixed rapidly on a vortex for 2 min, then passed through a prefiltration column (MiniPrefilter column #5188-2736; Agilent, Wilmington, DE) to remove any genomic DNA. RNA was then extracted according to the instructions for the RNeasy Mini Kit, including a 2 h DNase I treatment. For positive controls, RNA was extracted from approximately 100 mg of brain tissue using Tri Reagent (MRC, Inc., Cincinnati, OH) according to the manufacturer’s instructions.

To quantify DRK and K2P channel mRNA levels in fungiform taste buds, we used a two-tube RT-PCR assay with the PCR step conducted in a real-time thermal cycler (SmartCycler™, Cepheid, Sunnyvale CA). First-strand cDNA synthesis was performed using the OmniScript RT Kit (Qiagen) and qualitatively assessed on a BioAnalyzer (Agilent model 2100, Palo Alto CA). The maximum volume of taste RNA or 50 ng of brain RNA was used for the reaction in a total volume of 100 µl. Two microliters of cDNA was used for each qPCR reaction. The HotMaster Taq DNA polymerase kit (Eppendorf, Westbury NY) was used, with the following final concentrations: 1× reaction buffer, 3.5 mM Mg2+, 200 µM dNTPs, 300–900 nM sense and antisense primers, 200–250 nM fluorescent probes and 1.25 U HotMaster Taq. A 2-step PCR protocol was used for the DRK qPCR assays consisting of a 15 s denaturation step at 95 °C and 60 s annealing and extension at 60 °C. To quantify K2P expression, the following 2-step PCR protocols were used to amplify TRAAK, TREK-1 (15 s denaturation at 95 °C and 60 s annealing and extension at 64 °C) and TREK-2 (15 s denaturation at 95 °C and 60 s annealing and extension at 60 °C).

Using our established procedure [18,20], we employed a TaqMan (ABI) detection system in which the primer pairs for channel-specific sequences were multiplexed with the primer pairs for the housekeeping gene, GAPDH, for direct comparison of expression levels in the fungiform taste buds [21]. Channel-specific probes were labeled at the 5′-end with FAM as the reporter fluorophore and BHQ-1 at the 3′-end as the quencher. The GAPDH probe was labeled with ROX as the reporter fluorophore and BHQ-2 at the 3′-end as the quencher. The GAPDH probe was used to normalize the data. All DRK probes were obtained from Integrated DNA Technologies (Coralville IA) and their sequences and those of the accompanying DRK primers have been previously published [18]. The primer and probe sequences for the fatty acid sensitive K2P channels (TRAAK, TREK-1 and TREK-2) are listed in Table 1. All qPCR assays were carried out in triplicate and a minimum of three independent experiments was conducted.

Table 1

<table>
<thead>
<tr>
<th>K2P Channel</th>
<th>Genbank accession number</th>
<th>qPCR primers and fluoregenic probes</th>
<th>Corresponding nucleotide sequence</th>
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<tr>
<td>TRAAK</td>
<td>AF259502</td>
<td>Sense 5′-AATCTGGCTTTATCGAGGCTTGCC-3′</td>
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<td></td>
<td></td>
<td>Antisense 5′-GGCAAGCCGTCTTTGCGAAGG-3′</td>
<td>534–557</td>
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<td></td>
<td></td>
<td>Probe 5′-AGCGTTGCCGTTGGGTCACTC-3′</td>
<td>448–476</td>
</tr>
<tr>
<td>TREK-1</td>
<td>AF385402</td>
<td>Sense 5′-GCCCCTGTGCAACCATCATC-3′</td>
<td>907–924</td>
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<td></td>
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<td>Antisense 5′-GCCAGCCCAAGGAGAT-3′</td>
<td>1024–1043</td>
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<td></td>
<td></td>
<td>Probe 5′-TCATACCTGACCGACCATATTG-3′</td>
<td>932–957</td>
</tr>
<tr>
<td>TREK-2</td>
<td>NM_023096</td>
<td>Sense 5′-TCCTGGAACCTCCCGGAAGT-3′</td>
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<td></td>
<td></td>
<td>Antisense 5′-AGCAATAGAAACCCGAGA-3′</td>
<td>946–965</td>
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<td></td>
<td></td>
<td>Probe 5′-TGCCTTTCTGGCCGCGCTGCAAT-3′</td>
<td>837–861</td>
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<td>Sense 5′-TGCCACACCACTCAGCTTAC-3′</td>
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<td></td>
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<td>Antisense 5′-GGTGACAGGAGGATGTTGTC-3′</td>
<td>636–654</td>
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<td></td>
<td></td>
<td>Probe 5′-ATACGCCACACACCTCCAGAGG-3′</td>
<td>595–618</td>
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Two pore domain K+ (K2P) channel probes were labeled with the fluorescent reporter carboxyfluorescein fluorescent dye (FAM) on the 5′ end and the quencher, Black Hole Quencher-1 (BHQ-1) on the 3′ end. GAPDH probes were labeled with carboxy-X-rhodamine fluorescent dye (ROX) as the fluorescent reporter on the 5′ end and the quencher Black Hole Quencher-2 (BHQ-2) on the 3′ end.
For quantitative analysis, fluorescent signals in the samples were plotted against the respective qPCR cycle number. The cycle at which the growth curve crossed 30 fluorescent units was defined as the cycle threshold (\(C_T\)). This user-defined threshold was selected to occur during the log-linear phase of the growth curve, which is inversely proportional to the starting amount of target in the sample. Exact cycle thresholds were measured for each DRK or K2P channel as well as for the housekeeping gene, GAPDH. Delta \(\Delta C_T\) was calculated by subtracting the GAPDH \(C_T\) from the individual K+ channel \(C_T\). Comparing \(\Delta C_T\) values allowed for detection of relative transcript abundance between different sets of pooled taste buds by normalizing DRK or K2P channel expression to a constitutively expressed gene. Therefore, the smaller the \(\Delta C_T\), the greater the K+ channel expression. As previously described [18,20], for relative quantification of our samples, the arithmetic formula \(2^{-\Delta C_T}\) was used and takes into account the amount of target, normalized to an endogenous reference and relative to a calibrator. The DRK or K2P channel with the highest expression (or the lowest \(C_T\)) for each set of pooled taste receptor cells was defined as the calibrator for that set. The calculation of \(\Delta C_T\) involved subtraction of the \(\Delta C_T\) for each channel from the \(\Delta C_T\) calibrator value. The relative amount of target expression was determined according to the following relation [22]:

\[
\Delta C_T^1 = C_T^{\text{Channel}} - C_T^{\text{GAPDH}} \\
\Delta C_T^2 = C_T^{\text{CAL}} - C_T^{\text{GAPDH}} \\
\Delta C_T = \Delta C_T^2 - \Delta C_T^1 \\
\text{Relative expression} = 1/(2^{-\Delta C_T})
\]

where \(C_T\) is the cycle threshold for the DRK/K2P channels or GAPDH determined empirically; \(C_T^{\text{CAL}}\) is the cycle threshold for the calibrator, the most highly expressed channel in each assay. Mean relative expression values and standard deviations were calculated from the three individual sets of pooled taste bud types. To determine if there were significant differences among the expression of DRK channels in the fungiform taste buds of O–M and SSB rats multiplet pair-wise comparisons were made using a one-way ANOVA followed by Bonferroni’s post hoc test for significance (SPSS 13.0, SPSS Inc., Chicago, IL).

To determine if the efficiencies of the target and reference (GAPDH) amplification were consistent across template dilutions, we previously evaluated the \(\Delta C_T\) values for each set of DRK primers and GAPDH and K2P channels and GAPDH in three separate multiplexed reactions [18,20]. For each of the PCR reactions, the absolute value of the slope of the log input versus \(\Delta C_T\) was <0.1 demonstrating equal amplification efficiencies for the different starting template concentrations (data not shown). There was no effect on \(C_T\) values when the GAPDH primers were either limited or not limited in the reactions.

3. Results

3.1. Effects of fatty acids on fungiform taste receptor cells from O–M and SSB rats

To determine if the differences in dietary fat preference and the propensity to develop dietary-induced obesity were reflected in TRC responsiveness to fatty acids, we performed
Inhibition constants (IC\text{50}) were determined for the effective fatty acids by fitting DRK current inhibition data between 0.1 and 10 μM with a logistic function as previously described (Gilbertson et al., 1998). Percent (% inhibition was determined in 10 A\text{M} of each compound measured at the end of a voltage step from −80 to +40 mV. *Significantly different (p < 0.05) from O−M rats as determined by paired t-test. PUFA, polyunsaturated fatty acid; TG, triglyceride; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; tri-C18:2, trielnolein; tri-C18:3, trielnolein. Data represent averages from between 8 and 25 cells per value.

A series of experiments comparing the responses of isolated TRCs to a variety of fatty acids using patch clamp recording. Previously, in a preliminary study, we found that TRCs from obesity-resistant rats showed a greater inhibition by high concentrations (10 μM) of polyunsaturated fatty acids than those from an obesity-resistant strain (Fig. 1) although the inhibition constants (IC\text{50}) were identical for linoleic acid (~1 μM; 11). Following up on this initial report we tested a broader range of fatty acids and triglycerides to determine the specificity, total inhibition and IC\text{50} for each effective fatty acid. These data are summarized in Table 2. Similar to our initial study, only the cis-PUFAs were effective in blocking the delayed rectifying K\text{+} channels in fungiform taste cells from both strains. Concentration–response curves were generated for several cis-PUFAs between 0.1 and 10 μM to determine the IC\text{50} values. There were no significant differences among the individual values for different fatty acids within a species or for any fatty acid between species. However, for all cis-PUFAs, TRCs in the obesity-resistant S5B rats were more profoundly inhibited than those in O−M rats and this difference was statistically significant as determined by paired Student’s t-test comparing SSB and O−M rats (p < 0.05 for all cis-PUFAs; Table 1). Triglycerides, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were all ineffective in inhibiting DRK currents in fungiform TRCs from both strains of rats and there were no interstrain differences.

### Table 2
<table>
<thead>
<tr>
<th>Class</th>
<th>Fatty acid</th>
<th>O−M</th>
<th>SSB</th>
<th>O−M</th>
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<th>O−M</th>
<th>SSB</th>
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<td>1.00</td>
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<td>8.1</td>
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Inhibition constants (IC\text{50}) were determined for the effective fatty acids by fitting DRK current inhibition data between 0.1 and 10 μM with a logistic function as previously described (Gilbertson et al., 1998). Percent (%) inhibition was determined in 10 μM of each compound measured at the end of a voltage step from −80 to +40 mV. *Significantly different (p < 0.05) from O−M rats as determined by paired t-test. PUFA, polyunsaturated fatty acid; TG, triglyceride; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; tri-C18:2, trielnolein; tri-C18:3, trielnolein. Data represent averages from between 8 and 25 cells per value.

3.2. Linoleic acid enhances preference for subthreshold concentrations of saccharin in preference tests in obesity-resistant rats

We performed a series of two-bottle preference tests to determine if the differences in responsiveness of TRCs in SSB and O−M rats were correlated with any differences in behavioral responses to fatty acids. Since we have proposed that fatty acids act primarily as taste modulators rather than taste primers given their activity as open channel blockers of DRK channels [10,11,23], we tested the ability of linoleic acid to alter the preference for subthreshold concentrations of saccharin in 48-h two-bottle preference tests. Two groups of 8 rats of each strain were tested with either linoleic acid or lauric acid in the presence and absence of a subthreshold concentration of saccharin (0.5 mM). As shown in Fig. 2, linoleic acid or lauric acid were neither preferred nor avoided at 5 and 20 μM, two concentrations above and below the highest concentration of linoleic acid tested on isolated cells ([10,11]; see Table 2). At 0.5 mM, saccharin was also not preferred in these tests in either strain. However, addition of linoleic acid significantly enhanced preference for the saccharin solution in obesity-resistant (S5B) rats at both 5 (p = 0.023) and 20 μM (p < 0.01), while lauric acid had no effect, consistent with our electrophysiological observations (Fig. 1). Similar experiments in O−M rats failed to show a significant enhancement of preference for the mixture of linoleic acid and saccharin, although there was a trend toward this phenomenon at the higher linoleic acid concentration (2 μM; p = 0.068 by one-way analysis of variance).
ANOVA followed by Bonferroni’s post hoc test). Lauric acid presented alone or in a mixture with saccharin was neither preferred nor avoided by O–M rats.

3.3. Expression of DRK channels in O–M and S5B rats

Given our electrophysiological data, we have proposed the model shown in Fig. 3 to account for the differences between fatty acid signaling and DRK channels in S5B and O–M rats. Simply, both strains express fatty acid-sensitive (FA-s) and fatty acid-insensitive (FA-i) DRK channels in the membranes of TRCs. Since the IC50 for the effective fatty acids (PUFAs) is identical between the two strains (Table 2) but differ in the magnitude of inhibition (Fig. 1; [11]), we propose that the difference may lie in the ratio of expression of FA-s/FA-i DRK channels such that this ratio is greater in the obesity-resistant S5B strain. This would then translate into a greater fatty acid-induced activation of the cells in this strain to the same concentration of PUFAs.

To look for differences in DRK channels in TRCs, we compared the current densities of K+ and Na+ currents in S5B and O–M rats using patch clamp recording. There was no significant difference in the surface area of TRCs in S5B and O–M rats as determined by capacitance measurements (S5B: 10.5±1.8 pF; O–M: 9.9±2.4 pF; p=0.92 paired Student’s t-test). Consistent with our model, TRCs from O–M rats had a significantly greater K+ current density than those from S5B rats (p<0.01), although no differences were seen when comparing peak voltage-gated Na+ currents (Fig. 4). Moreover, no differences were noted in inwardly rectifying K+ currents between the two rat strains (data not shown).

We next turned to using quantitative real-time PCR to determine the source of differences in DRK currents in S5B and O–M rats. Using primer and probe sets for the nine DRK channel subtypes in multiplexed reactions with the housekeeping gene GAPDH [18], we measured relative expression for each DRK channel in S5B and O–M rats. As shown in Fig. 5, for several of the more highly expressed DRK channels (e.g., Kv2.2, Kv3.1 and Kv3.2), expression is greater in O–M than

![Fig. 3. Proposed model of DRK expression in obesity-resistant and obesity-prone rats to account for differences in fatty acid (FA) responsiveness. According to this model, S5B rats express a greater ratio of fatty acid-sensitive (FA-s) to fatty acid-insensitive (FA-i) DRK channels (top left) than O–M rats, which, in turn, have greater total DRK expression (top right). Upon exposure to polyunsaturated fatty acids (PUFA), a greater total DRK current inhibition would be predicted in TRCs from S5B rats (bottom left), while a larger residual K+ current would remain in the presence of PUFAs in TRCs from O–M rats (bottom right). Given the role of DRK channels in cell excitability, a larger subsequent electrophysiological response would be predicted from TRCs in S5B rats.](image)

![Fig. 4. K+ current density is greater in O–M rats. Mean current densities (pA/ pF) were estimated in fungiform taste receptor cells in O–M and S5B rats at +40 mV for steady-state K+ current (n=25 each) and at the peak inward Na+ current (n=12 each). Although there was no significant difference between O–M and SSB in terms of Na+ current density, O–M had a significantly greater outward K+ current density (p<0.01; Student’s t-test).](image)

![Fig. 5. Relative expression of nine DRK channels in fungiform taste receptor cells from SSB and O–M rats. Data are shown ± S.D. and represent the mean of three replicates each from a minimum of three independent experiments.](image)
in the S5B rats, which dovetails nicely with the differences in current densities shown in Fig. 4. Summing up the total relative expression of all DRK channels from the data shown in Fig. 5 using a common calibrator, DRK expression in O–M was 1.6 times greater than in S5B rats. The most highly expressed DRK channels (Kv1.5, Kv2.2, Kv3.1, Kv3.2) in both strains are similar to those found to be most highly expressed in anterior taste buds from Sprague–Dawley rats [18], suggesting that these channels may be commonly found in fungiform taste cells in a variety of strains. We [20] and others [24] have identified expression of several types of two-pore domain potassium (K2P) channels in rodent taste buds. Several members of this family of leak K+ channels are activated by cis-PUFAs [25] and, as such, may also be candidates that may have a role in the chemoreception of fat. QPCR assays on fungiform taste buds reveal high expression of one such member of the K2P family (TRAAK; KCNK4) and substantially lower expression of two others (TREK-1 and TREK-2; KNCK2 and KCNK10 respectively), yet there was no significant difference in expression of these channels in S5B and O–M rats (Fig. 6). Although we could see evidence of TRAAK-like currents in rat TRCs (inset, Fig. 6), our preliminary investigations suggest these channels are present functionally in less than 5% of fungiform TRCs by patch clamp recording (data not shown).

4. Discussion

In the present study, we have followed up on some early, more preliminary findings that demonstrated that there were functional differences in fatty acid responses of DRK channels in obesity-prone and obesity-resistant rat strains [11]. Using patch clamp recording coupled with quantitative PCR, we provide further evidence that the differences in fatty acid effects on DRKs in fungiform TRCs exist between S5B and O–M rats and that these differences may be attributable to underlying differences in expression of DRK channels, rather than a difference in the affinity of individual fatty acids for DRK channels. TRCs from obesity-prone rats express a higher concentration of total DRK channels consistent with electrophysiological recordings and this is especially evident in the expression of members of the Kv3 (Shaw) family of DRK channels.

4.1. Differences in fatty acid responses in obesity-prone and -resistant rats

S5B/Pl and O–M rats have been characterized as models for organisms that are resistant to dietary-induced obesity or prone to dietary-induced obesity, respectively. Numerous differences have been reported between these two strains including macronutrient preference [16], neuropeptide Y-mediated [26], orexigenic [27] and leptinergic pathways [28,29], and sympathetic nervous system activity [30]. Interestingly, Greenberg et al. [31] found that intraduodenal infusions of linoleic acid had a greater satiating effect in S5B rats than in O–M rats suggesting there were differences between how the two strains recognized and responded to dietary fat, which paralleled our initial work in the taste perception of fatty acids in these two strains [11].

In the present study we have shown that the chemoreception of fat (i.e., polyunsaturated fatty acids) is different in the taste systems of the S5B and O–M rat. For all PUFAs, DRK currents in fungiform TRCs were inhibited to a significantly greater degree in the obesity-resistant S5B rat. There were no
strain differences in responses of TRCs from fungiform TRCs to monounsaturated fatty acids, saturated fatty acids or triglycerides. Since DRK channels play roles in the repolarization of TRCs, one prediction from these data would be that the fungiform TRCs in SSB rats would be activated to a greater degree by PUFAs than those from O– M rats. To test this we performed two-bottle preference testing using linoleic acid, which targets DRK channels, and lauric acid, which has no effect of DRK channels in TRCs, to alter preference for a concentration of saccharin that was neither preferred nor avoided. While longer term preference tests such as these may be complicated by post-ingestive effects, it is nonetheless interesting that there were parallels between these behavioral data and patch clamp recording. First, PUFAs (e.g., linoleic acid) were able to enhance preference for 0.5 mM saccharin, a concentration that was not preferred, which would be predicted if PUFAs enhance stimulus-induced responses as has been reported (cf. Fig. 6 in [10]). Second, a saturated fatty acid, lauric acid, which has no effect of DRK channels in TRCs, did not alter saccharin preference. And, third, there was a greater effect in the obesity-resistant SSB rats that paralleled our findings using electrophysiological recording. Taken together, the results using this simple behavioral paradigm are consistent with the predictions based upon the effects of fatty acids on DRK currents in these rat strains. Clearly, however, more detailed behavioral assays looking at fat taste function in these two strains would be necessary to validate further this interpretation.

4.2. A link between DRK expression and fatty acid responsiveness in TRCs

The electrophysiological data suggested that it was not the affinity of the interaction between the fatty acid and DRK channel which varied between the two strains since the IC50’s were identical for all effective fatty acids in O– M and SSB rats. Rather, it appeared to us that the majority (~90%) of the DRK current was fatty acid-sensitive (FA-s) in the SSB rat, while in the O– M rat there was a significant proportion of the DRK current that was not inhibited by PUFAs, even at 10 μM. We could gain no insight into the properties of this fatty acid-insensitive (FA-i) current by analyzing the current in the presence and absence of PUFAs since these fatty acids act as open channel blockers [23] and profoundly change the kinetics of the DRK current in a time-dependent fashion [10]. As such, we could not infer the identity of the FA-i current directly by analyzing its kinetic properties and comparing it to known properties of heterologously expressed DRK channels [18].

To try and explore the underlying differences between DRK-mediated fatty acid responses in TRCs from O– M and SSB rats, we therefore performed a series of qPCR analyses on DRK expression in the anterior taste buds from these rats. The assays revealed that expression of DRK channels was generally greater in O– M rats, a finding that was supported by our electrophysiological data showing a greater density of DRK currents in this strain of rat. The differences between DRK expression was most evident for Kv3.1 suggesting that this channel may contribute to the phenotypic differences in DRK currents to a greater degree than other channel subtypes in fungiform taste buds. Unfortunately, the lack of a specific inhibitor for any member of the Kv3 (Shaker) family of DRK channels limits our ability to directly test the proportion of DRK current that is attributable to the Kv3.1 subtype, which would allow us to compare the magnitude of this current in each strain of rat.

To relate these expression data of DRK channels in the anterior tongue to the relative fatty acid responsiveness of fungiform taste buds requires that the relative fatty acid responsiveness of each DRK channel subtype be known. Unfortunately, at present, this is not the case. While the Kv1 (Shaker) family is clearly considered a fatty acid sensitive subtype based upon a number of studies [18,23,32,33], the case is less clear for the Kv2 and Kv3 families. Since we interpret our data as indicative of the FA-s/FA-i ratio being the difference between the O– M and SSB rats’ responsiveness to fatty acids, one might hypothesize that either the Kv2 and/or Kv3 subtypes represent the FA-i DRK channel population. If we assume that Kv1 represents the FA-s pool of DRK channels and [Kv2 + Kv3] the FA-i pool and sum up the total expression using data shown in Fig. 5, one generates an FA-s/FA-i ratio of 1.83 in SSB rats and 0.224 in O– M rats (Fig. 7A). On the other hand, if Kv3 channels are the only FA-i group of DRK channels, using the same approach would generate an FA-s/FA-i ratio of 5.74 in SSB rats and 1.00 in O– M rats (Fig. 7B). In either case, the FA-s/FA-i ratio is greater in SSB rats, consistent with our electrophysiological data. Of course, we are cognizant of the fact that there may be K+ channel expression differences other than those involving DRK channels that could also be contributing to the phenotypic differences between TRC responses in SSB and O– M rats.

Fig. 7. Hypothetical model of the ratio of fatty acid-sensitive to fatty acid-insensitive DRK channels in obesity-prone (OM) and -resistant (SSB) rat strains using the quantitative expression data generated in qPCR assays. (A) Relative total expression assuming Kv1 channels are fatty acid-sensitive and the Kv2 and Kv3 families are fatty acid-insensitive. (B) Relative total expression assuming Kv1 and Kv2 channels are fatty acid-sensitive and the Kv3 family is fatty acid-insensitive. Percentages refer to the percentage of total channel expression that is either FA-s (white) or FA-i (gray) determined from the data shown in Fig. 5. Number in parentheses refers to the ratio of FA-s/FA-i channels according to the assumptions above. In both models, the FA-s/FA-i ratio is higher in SSB rats and ranges from ~5 to 8 times greater in this obesity-resistant strain.
Of the four most highly expressed DRK channels in rat (Kv1.5, Kv2.2, Kv3.1, Kv3.2) in this study, only Kv1.5 has been unequivocally identified as being highly sensitive to extracellularly applied PUFAs. Our previous data [18] indicate that Kv1.5 is the major TRC fatty acid sensitive channel (and expressed in all fungiform TRCs) in Sprague–Dawley rats, which also show a high degree of PUFAs sensitivity in the anterior tongue. It has been reported that members of the Kv2 family are sensitive to extracellular free fatty acids (e.g., [33]). In addition, in a preliminary study looking at expression of Kv2.2 cDNA in a heterologous expression system (Chinese hamster ovary cells), we found that Kv2.2 was moderately sensitive to linoleic and arachidonic acid [34]. There is only a single report of a member of the Kv3 family being sensitive to fatty acids [35]. However, this study also suggested that the mechanism of inhibition of DRK currents by a PUFAs was markedly different in the Kv3.1 channel than it was in members of the Kv1 family. Another study argues that Kv3 channels are insensitive to PUFAs like arachidonic acid in a heterologous expression system [36]. Clearly, much more research is needed into the identity of the FA-s and FA-i DRK channel subtypes to validate this working hypothesis.

4.3. A model linking fat intake and peripheral fat chemoreception

Our results showing greater inhibition of DRK channels (i.e., activation of TRCs) in O–M rats, which prefer fat in macronutrient choice paradigms [16], are less responsive to PUFAs than those from S5B rats, which tend to eat much less dietary fat in the same assay. A model that takes into account the chemoreception of fat via DRK channels and its role in fat intake would thus be predicated on the assumption that input from pre- and post-ingestive chemoreceptors feed into the negative feedback pathways that contribute to the control of appetite. In such a model, the weaker chemosensory input seen in the O–M rat would thus be less capable of activating the pathways related to the cessation of fat (i.e., food) intake, resulting in a greater relative intake of fat. Although speculative, the results of Greenberg et al. [31], showing that fatty acid mediated inhibition of sham feeding was weaker in O–M rats than it was in S5B rats, would agree in principle with this model. For example, the weaker activation of the chemoreceptive enterodendocrine cells in the small intestine of O–M rats during fat feeding would be predicted to release less of the hormone cholecystokinin (CCK), which is linked with meal termination (for review, see [37–39]), compared to an S5B rat. It is clear that free fatty acids are capable of stimulating the release of CCK from enterodendocrine cells [40,41] and we have shown that an enterodendocrine cell line, STC-1, responds to PUFAs in a manner similar to TRCs using patch clamp recording [42]. The taste system’s role thus may be to either contribute to or be reflective of these differences in S5B and O–M rats. Interestingly, however, a recent report in mice suggested the opposite relationship—that greater sensitivity to macronutrients like sweet and fat more profoundly stimulates intake [43]. It should be noted although that in this study, solution preferences were taken as an indicator of orosensory responsiveness to fat, which has never been demonstrated unequivocally to be the case. Nonetheless, the role of chemosensory input both pre- and post-ingestively in the control of food (fat) intake is one that warrants greater investigation.

References


[42] Kim I, Liu L, Gilbertson TA. Inhibition of K⁺ channels by fatty acids may represent a common mechanism for the chemoreception of fat in both pre- and post-ingestive targets. Chem Senses 1998;23:612.