Assessment of the presence of chemosensing receptors based on bitter and fat taste in the gastrointestinal tract of young pig

M. Colombo, P. Trevisi, G. Gandolfi, and P. Bosi

University of Bologna, DIPROVAL, 42123 Reggio Emilia, Italy

ABSTRACT: Knowledge on porcine bitter and fat taste receptors and on their expression in gastrointestinal tract of pigs is scarce. We searched for the presence of porcine homologous sequences for 13 human transcripts of bitter and fat taste receptors in ENSEMBL and National Center for Biotechnology Information databases. For taste 2 receptor (TAS2R) 8, alignment was not observed; for TAS2R13 and TAS2R46 the porcine predicted sequence aligned with several other human bitter genes. For 7 genes for bitter taste (TAS2R1, TAS2R3, TAS2R7, TAS2R9, TAS2R10, TAS2R16, and TAS2R38) and for 3 genes for fat taste (GPR40, GPR43, and GPR120), a full homology for exon sequences was found and primers were designed by Primer3. These 7 genes were amplified with real-time PCR and verified on agarose gel in 5 gastrointestinal segments of weaned pigs: oxyntic (ST1), pyloric (ST2), and cardiac to oxyntic transition mucosa (ST3), jejunum (JEJ), and colon (COL). Suitability of mRNA was verified by amplifying RPL4 and HMBS2 genes. Each bitter taste gene was detectable on agarose gel in at least 1 subject of all the gastrointestinal segments except for TAS2R3 and TAS2R38 that were never detected in ST1 and COL, respectively. The inspection of bitter taste genes amplification curve indicated that the expression was in general very low. GPR43 and GPR120 were present in all segments from all pigs. Expression was not detected for GPR40. Data also indicate that colon is the preeminent tract where fat detection by GPR120 takes place (P < 0.001). The presence of gene expression for several chemosensing receptors for bitter and fat taste in different compartments of the stomach confirms that this organ should be considered a player for the early detection of bolus composition.

Key words: bitter, fat, pig, stomach, taste receptor


INTRODUCTION

Humans recognize sweet, umami, sour, salty, and bitter, and novel tastes have been described such as fat taste and metallic (Chaudhari and Roper, 2010). Recent research indicates that the presence of chemosensing receptors for bitter and fat taste is not restricted to the mouth but is extended to other organs and tissues. Particularly, their location along the whole digestive tract contributes to the control of secreting activity, regulation of several hormones, and afferent neuronal modulation. In pigs, the taste chemosensory system has been investigated for its impact on the feed preference and feed intake (Roura and Tedò, 2009). The diffuse gastrointestinal chemosensory system consists of solitary chemosensory cells that express molecules of the chemoreceptorial cascade activated by G protein-coupled receptors (GPR) and α-gustducin interaction (Iwatsuki and Torii, 2012). Taste 2 receptors (TAS2R) are the GPR identified as receptor for bitter taste. Bitter taste is initiated by a large number of different and unrelated organic molecules recognized by a broad range of receptors of the TAS2R family; humans have 25 functional bitter taste receptors genes. Other GPR have been identified in taste buds and likely contribute to the detection of nutrients. These include GPR40, GPR43, and GPR120 genes, which are expressed in subsets of taste cells and detect fatty acids (Cartoni et al., 2010). To date, we are not aware of any study that investigated TAS2R or GPR genes in pigs.

The goal of this study was to assess the presence of porcine homologous sequences for the known human transcripts of bitter and fat taste receptors in different segments of the gastrointestinal tract of the young pigs.

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2Corresponding author: paolo.bosi@unibo.it
Table 1. Frequency of pigs with expression of TAS2R genes in 5 gastrointestinal points

<table>
<thead>
<tr>
<th>Gene</th>
<th>ST1</th>
<th>ST2</th>
<th>ST3</th>
<th>JEJ</th>
<th>COL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS2R1</td>
<td>4/5</td>
<td>4/5</td>
<td>4/4</td>
<td>3/5</td>
<td>2/5</td>
</tr>
<tr>
<td>TAS2R3</td>
<td>0/5</td>
<td>2/5</td>
<td>3/4</td>
<td>1/5</td>
<td>3/5</td>
</tr>
<tr>
<td>TAS2R7</td>
<td>3/5</td>
<td>4/5</td>
<td>3/4</td>
<td>2/5</td>
<td>2/5</td>
</tr>
<tr>
<td>TAS2R9</td>
<td>4/5</td>
<td>4/5</td>
<td>4/4</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td>TAS2R10</td>
<td>4/5</td>
<td>4/5</td>
<td>4/4</td>
<td>4/5</td>
<td>1/5</td>
</tr>
<tr>
<td>TAS2R16</td>
<td>1/5</td>
<td>1/5</td>
<td>1/4</td>
<td>5/5</td>
<td>3/5</td>
</tr>
<tr>
<td>TAS2R38</td>
<td>2/5</td>
<td>1/5</td>
<td>2/4</td>
<td>1/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

1Oxyntic (ST1), pyloric (ST2), and cardiac to oxyntic transition mucosa (ST3), jejunum (JEJ), and colon (COL).

Table 2. Effect of the point of measure on the expression of GPR43 and GPR120 genes

<table>
<thead>
<tr>
<th>Point of measure</th>
<th>ST1</th>
<th>ST2</th>
<th>ST3</th>
<th>JEJ</th>
<th>COL</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR43, normalized expression</td>
<td>0.025</td>
<td>0.036</td>
<td>0.038</td>
<td>0.060</td>
<td>0.063</td>
</tr>
<tr>
<td>GPR120, normalized expression</td>
<td>0.017</td>
<td>0.009</td>
<td>0.012</td>
<td>0.004</td>
<td>0.081</td>
</tr>
</tbody>
</table>

1Oxyntic (ST1), pyloric (ST2) and cardiac to oxyntic transition mucosa (ST3), jejunum (JEJ), and colon (COL).

MATERIAL AND METHODS

We searched for the presence of porcine homologous sequences for 13 human transcripts of bitter and fat taste receptors in ENSEMBL and National Center for Biotechnology Information databases. Genes for which a full homology for exon sequences was found in the pig were tested for their expression in the gastrointestinal tract. The animal protocol was approved by the deputed committee of our university.

The following 5 gastrointestinal segments were obtained after euthanasia from 5 Large White castrated male pigs (42 d of age; 11.8 ± 0.33 kg average BW): oxyntic (ST1), pyloric (ST2), and cardiac to oxyntic transition mucosa (ST3), jejunum (JEJ; at 75% of length) and ascending colon (COL; near the centrifugal turns). Prior to the sacrifice the pigs were receiving a typical postweaning diet based on cereals (extruded in parts), cereal byproducts, milk whey, and soybean (Glycine max) meal. The tissues were immediately snap frozen in liquid N and preserved at –80°C until analysis. Total RNA was isolated according to Takara Fast Pure kit (Takara Bio Inc, Otsu, Japan) protocol and reverse transcribed using the ImProm-II Reverse Transcription System (Promega, Madison, WI). The following primers were designed using Primer 3 (http://frodo.wi.mit.edu/primer3/) on pig sequences TAS2R1 [forward (F): ATCCCTCACCCAATCTTCTC; reverse (R): TCCATTACGTTTGCTCTGGI], TAS2R3 (F: CCCATCTCATTCTCTGG; R: ATCCACCGATTTCCACTGAG), TAS2R7 (F: ATGCAATTTACATGCCCACAG; R: AGCTGGAGGTGGCTACAAAGA), TAS2R9 (F: CAAGCCTTACATTCATCTC; R: TGTCTCCTCTTTTGTGGAAA), TAS2R10 (F: TTTGCTCAATCTGGGTGTC; R: CTTGCTGGGTGTCTCTGAT), TAS2R13 (F: CCAGGACCAAGGCCACATA; R: TCAATGCATTCAACAAGCA), TAS2R16 (F: ACCGTGACGGAGAGGCTG; R: ACGTGCTGGGACAAATGAGG), GPR120 (F: CCAATGGTGTGAGAAAATC; R: TGATGCCTCTGGATCTGTGTA), GPR40 (F: GCATCAAACGCAGCAGCTCAAT; R: TCCITTGCTCTTTCTGCT), and GPR43 (F: CTCATGGTTTCCGCTCT; R: GCAGCAATCACTCCGTACAA).

Amplification was performed in a LightCycler Real-Time PCR Systems (Roche Applied Science, Mannheim, Germany) by a shuttle PCR (2 steps) in a 10-μL volume containing 2 μL of cDNA, 5 pmol of each primer, and 5 μL of SYBR Premix Ex Taq II (Perfect Real Time, Takara Bio Inc). Amplifications consisted of 40 cycles at 95°C for 5 s and 60°C for 22 s. The specificity of each amplification was checked by melting curve analysis. For GPR43 and GPR120 genes an absolute quantification was performed; afterwards the data were normalized by geometric mean of the absolute expression of the two housekeeping genes hydroxymethylbilane synthase (HMBS2) and ribosomal protein L4 (RPL4) obtained with the primer pairs F: AGGATGGGCAACTCTACCTG and R: GATGGTGGCCTGCATAGTCT and F: CAAGAGTAACTACAACCTTC and R: GAACTCTACGATGAATCTTC, respectively. The quantification of TAS2R genes was not possible due to low gene expression; therefore, the analysis was limited to determining the presence or absence of the specific amplicon. Then 10 μL of the PCR reaction were migrated in 1.5% Tris-borate-EDTA agarose gel to check the product size of the fragment.

Data of gene expression were analyzed by ANOVA using the MIXED procedure (SAS Inst., Inc., Cary, NC) with testing point in the gastrointestinal tract as repeated measure in each pig. Statistical significance was set to P < 0.05. The Tukey test for multiple contrasts was used.
RESULTS AND DISCUSSION

The in silico analysis showed that for TAS2R8 alignment from human to pig was not present. For TAS2R13 and TAS2R46 the porcine predicted sequence also aligned with several other human bitter genes. For 7 genes for bitter taste (TAS2R1, TAS2R3, TAS2R7, TAS2R9, TAS2R10, TAS2R16, and TAS2R38) and for 3 genes for fat taste (GPR40, GPR43, and GPR120), a full homology for exon sequences was found. Each bitter taste gene was detectable on agarose gel in at least 1 subject of all the gastrointestinal segments except for TAS2R3 and TAS2R38 that were never detected in ST1 and COL, respectively (Table 1). Conversely, TAS2R1, TAS2R9, and TAS2R10 were present in all tested ST3 samples and TAS2R16 in all JEJ samples. The inspection of bitter taste genes amplification curve indicated that the expression was in general very low; hence, it was not possible to perform a quantitative analysis.

Putative receptors for bitter molecules also are diffused in the porcine gastrointestinal tract, similar to observations in mouse (Mus musculus) and rat. Concerning the stomach, the identification in pig gastric tissues of some of the tools to translate the taste signals (Widmayer et al., 2011) can be put in connection with the presence of transcripts for some TAS2R as well as of other taste receptors. Particularly, TAS2R in the stomach may participate in the control of gastric emptying in the pig, as suggested in mice (Janssen et al., 2011).

The GPR43 and GPR120 transcripts were detected in all the segments whereas no expression was found for GPR40. For GPR43, differences were not observed among the gastrointestinal segments whereas GPR120 had increased expression in the colon compared to each of the other segments (P < 0.001) (Table 2). Our observation of higher gene expression of GPR120 in the colon, as compared with small intestine, agrees with the major role of GPR120 in the colon in the incretin rise after stimulation with fatty acids in mice (Hirasawa et al., 2004). Furthermore, this response of incretin was not seen for GPR40. Conversely, the possible activation of GPR43 and GPR120 in different gastric areas has never been fully described.

In conclusion, chemosensors for bitter and fat tastants contribute to the diffuse chemosensory system in the gut and also provide the basis for further studies aimed at assessing the potential genetic variability in pigs and the screening of potential adjuvant or masking molecules.

LITERATURE CITED


