Nonruminant Nutrition Symposium: Nutrient and Non-Nutrient Sensing and Signaling in the Gastrointestinal Tract

1115 Bitter taste receptors and gastrointestinal chemosensing. C. Sternini*[1], H. E. Raybould[2], L. M. Rinaman[3], and E. Rozegurt[1], [1]*UCLA, School of Medicine*, *Los Angeles, CA,* [2]*UC Davis, School of Veterinary Medicine*, *David, CA,* [3]*University of Pittsburgh*, *Pittsburgh, PA*.

Bitter taste has evolved as a warning signal against the ingestion of toxins, playing a crucial role in survival. The gastrointestinal (GI) lumen lining is exposed to nutrients and non-nutrients and might use the same mechanisms mediating oral taste signaling to detect harmful substances. This is supported by the discovery that signaling molecules transmitting bitter taste in the tongue are expressed in the GI mucosa, including the bitter taste receptors family, T2Rs, and the heterotrimeric G-protein subunits, α-gustducin (αGust) and α-transducin (αTrans). We identified transcripts for T2Rs, αGust and αTrans in the mammalian GI mucosa and in enteroendocrine STC-1 cell line, by RT-PCR. We showed that αGust and αTrans immunoreactivities (IR) are localized to set of enteroendocrine cells producing CCK, GLP1, PYY or ghrelin, peptides involve in food intake and GI function. T2R138-IR, the phenylthiocarbamide receptor, is also localized to GI epithelial cells. Intragastric administration of T2R agonists in rodents activates neurons in the brainstem via a vagal pathway. Selective T2R agonists inhibit food intake when administered intragastrically and induce conditioned flavor avoidance as measured with the 2-bottle choice paradigm, suggesting that GI T2Rs represent a second line of defense in the lumen. T2R agonists induce increase in intracellular calcium and CCK release in STC-1 cells and intraluminal T2R ligands significantly increased pCAMKII-IR, a marker of cell activation, in duodenal CCK cells and in the nodose ganglia. This suggests that T2R activation of enteroendocrine cells release CCK and activate vagal neurons. Overall, these findings support the concept that the GI mucosa lining is equipped with a chemosensory machinery for the detection of harmful substances, including food-borne toxins, drugs, and bacteria. We propose that activation of GI T2Rs by toxins induces a cascade of events triggering intracellular calcium increase and release of signaling molecules from enteroendocrine cells that in turn activate neuronal pathways responsible for initiating a protective response to guard the body from environmental hazards.

Key Words: enteroendocrine cells, vagal afferents

1116 T1R-mediated taste transduction mechanisms. S. C. Kinnamon*, *University of Colorado Denver*, *Aurora*.

Taste buds in the oral cavity are the chemosensory end organs that guard the entrance to the alimentary canal. Each taste bud comprises approximately 50–100 individual taste cells that detect the chemicals that elicit the sweet, salty, sour, bitter, and umami (glutamate) taste qualities. During the past decade, considerable progress has been made in identifying the receptors involved in the transduction of each taste quality. Ion channels are involved in the detection of salts and acids, while G protein coupled receptors (GPCRs) and second messengers mediate the transduction of bitter, sweet, and umami taste stimuli. Two classes of GPCRs have been identified, the T2R bitter receptors and the T1R receptors for sweet and umami stimuli. Three T1Rs have been identified, T1R1, T1R2, and T1R3. T1R3 combines with T1R1 (T1R1/T1R3) to bind glutamate and 5′-ribonucleotides, while T1R3 combines with T1R2 (T1R2/T1R3) to bind sugars, synthetic sweeteners, and sweet proteins. These receptors share similar downstream signaling effectors. The canonical transduction pathway involves Gβγ activation of PLCβ2, production of the second messengers IP3 and diacylglycerol, release of Ca^{2+} from intracellular stores, and Ca^{2+}-dependent activation of a monovalent-selective cation channel, TRPM5. These events lead to membrane depolarization and release of ATP as a transmitter to activate gustatory afferent nerve fibers. Genetic knockout of PLCβ2, IP3R3, and TRPM5 each severely compromises both sweet and umami taste responses, validating their central role in the transduction process. The Gα that mediates sweet and umami transduction is Gα-gustducin, which activates PDE to decrease intracellular cAMP. Although the exact role of cAMP in transduction is not clear, gustducin knockout mice have reduced responses to both sweet and umami stimuli. My lab has shown recently that α-gustducin knockout mice have elevated cAMP levels in taste buds, suggesting that gustducin is tonically active in the absence of taste stimuli. We suggest that α-gustducin tonically activates PDE to keep cAMP levels low and prevent chronic adaptation to taste stimuli.

Key Words: gustducin, T1R3, TRPM5

1117 Gut sensors for spices and odorants. T. Braun[1], P. Voland[2], L. Kunz[1], C. Prinz[2], and M. Gratzl*[1], [1]*Institute of Anatomy, Ludwig Maximilian University Munich*, *Munich, Germany*, [2]*II. Med. Dept., Technical University Munich*, *Munich, Germany*.

Enterochromaffin cells release serotonin in response to mechanical stimulation or in response to certain nutrients in the lumen of the intestine. The secreted serotonin then stimulates sensory components of the enteric nervous system, ultimately controlling gut peristalsis as well as water and chloride transport by enterocytes. Microarray gene chip data suggested that enterochromaffin cells might express olfactory G-protein-coupled receptors that are typically found in the nose. Laser capture-microdissected human intestinal enterochromaffin cells and a cell line derived from human enterochromaffin cells were found to express the same 4 olfactory receptor genes by RT-PCR: OR73, hOR17–7/11, OR1G1, and hOR17–210. Thymol, which binds to OR1G1, is a component of thyme spice. Thymol triggers a transient rise in intracellular calcium and a dose-dependent increase in serotonin release, whereas phenol does not. Other odorant ligands showed similar responses: eugenol and isoeugenol (binds OR73), methylsalicylate (receptor unknown), geraniol (binds hOR17–7/11 and OR1G1), bourgeonal (binds hOR17–7/11 and hOR17–4), and helional (binds hOR17–7/11 and hOR17–40) increased intracellular calcium, and stimulated serotonin release by exocytosis. Our study indicated that enterochromaffin cells express olfactory receptors that may be stimulated by odorant ligands in the intestinal lumen to release serotonin. The results suggest that luminal odorants may influence gut motility and secretion.

1118 Amino acid sensing in the gut epithelium. D. G. Burrin* and B. Stoll, *USDA Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX.*

Amino acids are elemental substrates for cellular protein synthesis in the body. Amino acids are present in the diet and absorbed across the intestinal epithelium into the portal blood by a family of transport proteins that are specific for different structural properties of amino acids. Our previous work has shown that dietary amino acids are also extensively metabolized by the gut epithelium and in the case of glutamate, used as a major oxidative fuel. However, recent evidence shows that amino acids are more than just nutrients or metabolic substrates, but also functions as signaling molecules in intestinal physiology and cell metabolism. The recent discovery of novel proteins reveals the molecular mechanism of

how gut epithelial cells "taste" or recognize specific amino acids. One such group of proteins is the taste receptors, which is a small family of 3 G-protein-coupled receptors (T1R1, T1R2 and T1R3) that form heterodimeric complexes that bind glutamate and sugars. Another more well known group of proteins, the metabotropic glutamate receptors are also expressed throughout the gastrointestinal tract. Both the taste and metabotropic receptors are localized on epithelial, endocrine and neuronal cells and when activated stimulate signal processes that are involved in gastric emptying, secretion and insulin release. Dietary glutamate increases vagal afferent nerve activity in the brain and is blocked by cholinergic inhibitors suggesting that these processes are mediated via extrinsic nerve pathways. Arginine is an amino acid that is conditionally essential in the neonate and can be extensively metabolized in the intestine. Recent studies suggest that arginine specifically stimulates protein synthesis in intestinal epithelial cells via activation of mammalian target of rapamycin (mTOR) and downstream signaling targets, p70S6 kinase and 4EBP-1. The mechanism whereby arginine activates mTOR does not involve production of nitric oxide, a key signaling product of arginine metabolism. The presentation will discuss these 2 examples of amino acid sensing mechanisms in the gut and the implications for animal growth and development.

Key Words: glutamate, arginine, enteroendocrine cells

1119 Nutrient sensors expressed in gut enteroendocrine cells regulate nutrient-responsive secretion of satiety hormones. S. Shirazi-Beechey*, K. Daly, A. Moran, and J. Dyer, *University of Liverpool*, *Liverpool, UK*.

The gastrointestinal (GI) tract is a sensory organ that responds to signals arising in its lumen. Intestinal nutrient sensing plays an important role in controlling GI function including regulation of gastric emptying, gut motility and nutrient absorption. In addition, molecular events in the lumen of the GI tract induce satiety hormone release from enteroendocrine cells that lead to activation of systemic, hormonal and/or neural pathways involved in the regulation of food intake and appetite. I shall describe mechanisms by which the intestinal epithelium senses nutrients i.e., sugars and short chain fatty acids resulting in secretion of gut hormones that regulate nutrient absorption and food intake. We showed that the sweet taste receptor (T1r2 + T1r3) and its coupled G-protein, gustducin, are expressed in intestinal enteroendocrine cells and act as the intestinal glucose sensor. We then demonstrated that dietary sugars and artificial sweeteners increase expression of intestinal glucose absorptive capacity in wild type mice, but not in T1r3 or gustducin knockout mice. In addition it has been shown that these knockout mice have deficiencies in secretion of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) in response to orally ingested carbohydrate, or when glucose was directly administrated into the intestinal lumen. Such data indicate that sweet taste receptor in intestinal enteroendocrine cell detects extracellular sugar and then responds with secretion of gut hormones. It is proposed that dietary fiber can increase GLP-1 release providing potential mechanisms for the stimulation of satiety and suppression of hunger. Dietary fiber is fermented in the large intestine to short chain fatty acids, acetate, propionate and butyrate. It has been shown that GPR41, and GPR43 may act as the SCFA sensor. Work in our laboratory has shown that these GPRs are expressed in the large intestinal endocrine cells. Furthermore exposure of L-type enteroendocrine cells to either SCFA or a compound known to specifically activate GPR43 results in secretion of GLP-1.

Key Words: nutrient sensing, intestine, GLP-1

1120 Effect of artificial sweeteners on the expression of swine intestinal Na^+/glucose co-transporter 1, SGLT1. A. Moran*[1], D. Batchelor[1], C. Ionescu[2], D. Bravo[2], and S. Shirazi-Beechey[1], [1]*University of Liverpool*, *Liverpool, UK*, [2]*Pancosma*, *Geneva, Switzerland*.

A shorter suckling period in piglets leads to several disorders including nutrient malabsorption, diarrhea, malnutrition and dehydration. We have shown that a low concentration of a combination of artificial sweeteners, saccharin and neohesperidin dihydrochalcone (Sucram) included in piglets' diets enhances the expression of intestinal SGLT1 and the gut capacity to absorb glucose. We determined previously that the sweet taste receptor comprised of T1R2+T1R3 is expressed in intestinal enteroendocrine cells and that dietary sugars and artificial sweeteners act in the intestine, on the sweet taste receptor, and its coupled G-protein gustducin, to elicit upregulation of SGLT1. Our studies in mouse intestine showed that only those artificial sweeteners that activated mouse lingual epithelium sweet taste receptor led to upregulation of intestinal SGLT1. Aim: to determine effects of sucralose, cyclamate, aspartame and acesulfame K (aceK) on intestinal SGLT1 expression. Five groups of 28 d old piglets (n = 8 per group) were weaned onto a commercial diet lacking any artificial sweeteners, with their drinking water containing either no sweetener, sucralose (2 mM), cyclamate (10 mM), aspartame (1 mM) or aceK (10 mM). All consumed the same amount of food and water. Subsequently, they were sacrificed by euthanasia under ethical permission. SGLT1 expression was assessed at levels of mRNA, protein and function. Results: there was a 2.73- ($P = 0.003$), 3- ($P < 0.0001$) and 2.8- ($P = 0.0005$) fold increase in SGLT1 expression in response to inclusion of sucralose, cyclamate and aspartame in the drinking water respectively compared with that in the control. AceK had no effect on SGLT1 expression. Results indicate that intestinal sweet taste perception in pig is different to some other species. These are due to sequential differences in amino acid residues of sensor proteins interacting with artificial sweeteners, the knowledge of which is essential for designing species-specific sweeteners. The data also highlight the potential of using other artificial sweeteners, having assessed the lowest effective concentrations, as food supplements.

Key Words: SGLT1, artificial sweetener, piglet's diet