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Intestinal enteroendocrine L cells in amino acid sensing and diseases

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1. ABSTRACT

Enteroendocrine L cells are open-type enteroendocrine cells that play an important role in amino acid sensing. They detect amino acids by a number of membrane receptors such as calcium-sensing receptor and G protein coupled receptor family C group 6 subtype A. The receptors activate signaling pathways and trigger cellular electrical activities, inducing gut hormones secretion (glucagon-like peptide 1, glucagon-like peptide 2 and peptide YY). This review focuses on an array of findings on L cells as models, receptors and signaling pathways, electrical activities and hormones secretion in amino acid sensing. Several diseases that are closely related to L cells are also reviewed.

2. INTRODUCTION

Enteroendocrine cells (EECs) are specialized intestinal epithelial cells widely distributed in the intestinal tract (1, 2). Although EECs account for less

than 1% of the total intestinal epithelium cells, they form the largest endocrine organ in the body (3). EECs are primary chemo-sensors in the intestinal lumen collecting and integrating information, releasing signaling molecules, activating nerve fiber and responding to luminal contents (4).

Enteroendocrine L cells are open-type EECs with apical processes facing the gut lumen in nutrient sensing (5). They are distributed along the length of intestinal epithelium, but the colon harbors the highest density (6). L cells are responsive to a range of luminal components, particularly the digestion products of protein, carbohydrates and fats (7, 8). For example, amino acids, such as glutamine, have been shown to trigger membrane depolarization and electrical activity in L cells that activate signaling pathways and stimulate gut hormone secretion (9). In response to nutrients stimulation, L cells secrete gut hormones such as glucagon-like peptide 1 (GLP-1), glucagon-like peptide 2 (GLP-2) and peptide YY (PYY) (10). These hormones regulate nutrient absorption and energy homeostasis in many ways (11, 12).

3. L CELL MODELS

3.1. Primary intestinal enteroendocrine cell

Primary intestinal EEC is a common model for intestinal nutrient absorption experiments *in vitro* (13). Embryonic rat intestinal cells, for example, secrete hormones in response to various extracellular regulatory factors but fail to response to glucose because the corresponding receptors are not expressed in embryonic L cells (14). In addition, co-culture of various adult mouse primary intestinal cells harboring L cells *in vitro* has been explored (15).

3.2. STC-1 cell line

STC-1 cell line was derived from a duodenum tumor of double transgenic mice harboring the minigene of the rat insulin promoter that drives the expression of the simian virus 40 large T antigen and the polyomavirus small T antigen. Originally, STC-1 cells have been used as a model of native CCK-producing I cells (16) as well as EECs differentiation (17), cellular signaling mechanisms involved in gut hormones secretion (18, 19), tumor cell growth (20) and intestinal immune responses (21).

3.3. GLUTag cell line

GLUTag cell line was derived from a colonic tumor of a transgenic mouse expressing simian virus 40 large T antigen under the control of the proglucagon promoter (22). This cell line has been shown to secrete GLP-1 in response to a range of physiological stimuli including monosaccharides, amino acids and fatty acids through protein kinase A (PKA) or protein kinase C (PKC) pathway (23-25). However, GLUTag does not show the polarity of an L cell, such as apical processes facing gut lumen. As such, results concluded from GLUTag might be difficult to reflect what happened in natural cells.

3.4. NCI-H716 cell line

NCI-H716 cell line was derived from a poor differentiated adenocarcinoma of human cecum and has shown some endocrine properties such as GLP-1 secretion and expression of chromogranin and glucagon (26). NCI-H716 secretes GLP-1 in response to fatty acids, cholinergic agonists, glucose-regulated protein, and PKA and PKC activators (27). However, these activators are unable to regulate expression of proglucagon. For example, PKA up-regulates proglucagon expression in animal models but fails to regulate it in NCI-H716 (28). Thus, the reliability of NCI-H716 as a model of human L cells remains to be proved.

4. AMINO ACID SENSING RECEPTORS IN L CELLS

Amino acid sensing in L cells relies on a number of membrane receptors. These receptors, with specific recognition of some amino acids, can activate signaling pathways, trigger cellular electrical activities and induce gut hormones secretion (Figure 1). Here, we summarize three important amino acid sensing receptors.

4.1. Calcium-Sensing Receptor

Calcium-Sensing Receptor (CaSR) is a typical G protein-coupled receptor (GPCR) belonging to group II of family C (29). It has been firstly cloned from bovine parathyroid cells by Brown and coworkers in 1993 (30). CaSR is known as seven-transmembrane domain receptor that exists in the form of dimer onto the cell membrane. The most studied roles of CaSR is homeostatic maintainer of systemic calcium (31). It can sense imperceptible change of extracellular calcium. The CaSR is widely expressed, includes L cells, where it is reported to regulate secretion of satiety hormones (32, 33). In the L cells, CaSR has been reported to act as a nutrient (amino acids / glucose) sensor, monitoring, and coordinating digestion, absorption and secretion (31, 34, 35). For example, rat intestinal L cells recognize *L*-amino acids by CaSR, especially *L*-aromatic amino acids, and secrete gut hormones (32, 36). This process seems to involve depolarization of the plasma membrane (9). In the STC-1 model, CaSR activates phospholipase C (PLC) and inositol triphosphate (IP3) signaling pathways after sensing amino acids that induces Ca^{2+} release from endoplasmic reticulum and extracellular Ca^{2+} entering the cell due to the activation of TRPC and *L*-VDCC (37). As a consequence, intracellular calcium

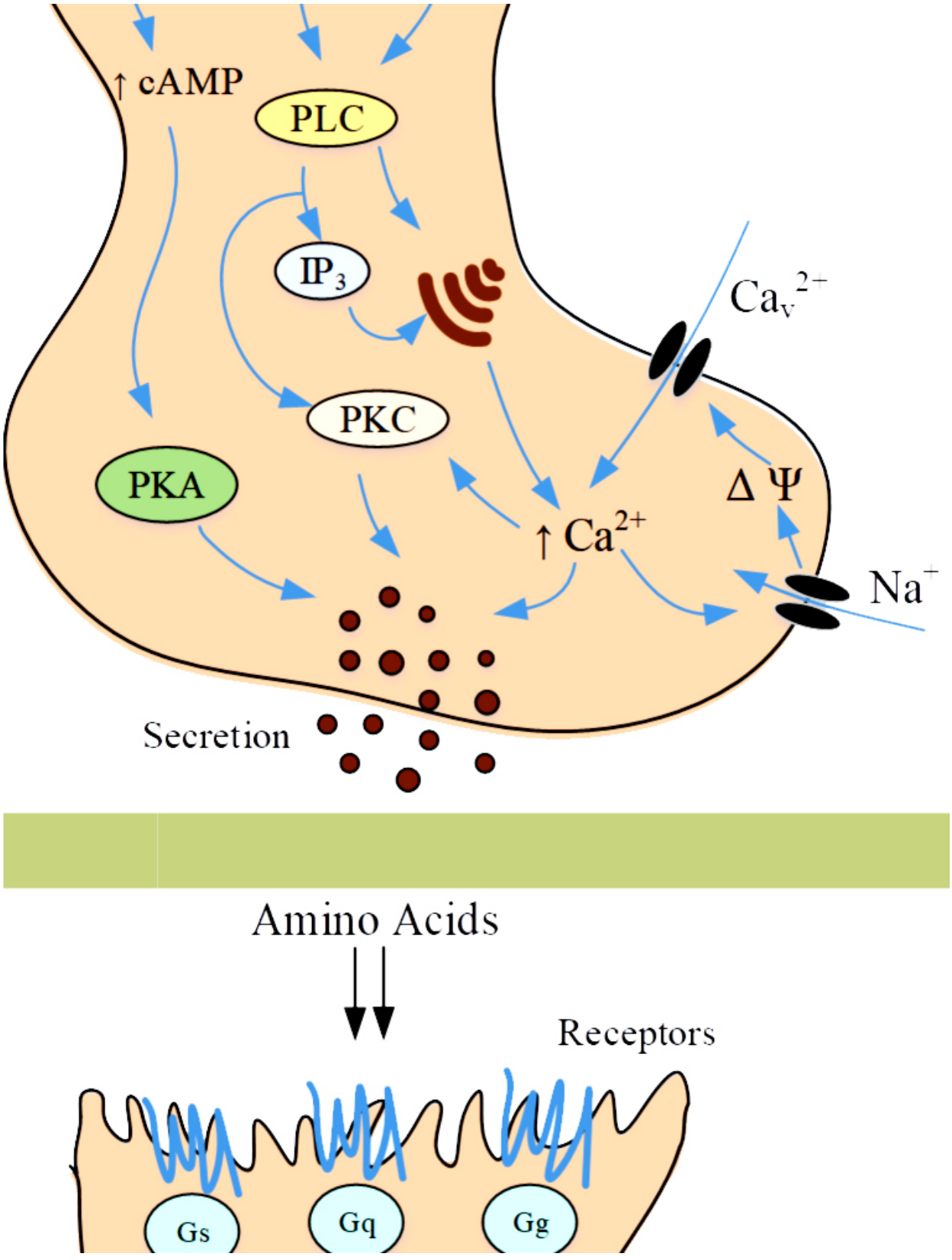

Figure 1. Amino acid sensing in L cells

concentration increases, which stimulates exocytosis of CCK and GLP-1 (38). Thus, CaSR induces increase of intracellular Ca^{2+} and stimulates hormone secretion by activating downstream signaling pathways and ion channels when sensing amino acids.

4.2. G protein coupled receptor family C group 6 subtype A

G protein coupled receptor family C group 6 subtype A (GPRC6A) is another important amino acid sensing receptor (39). Unlike CaSR, GPRC6A is not very sensitive to *L*-aromatic amino acids, but can be activated by basic amino acids including *L*-arginine, *L*-lysine and *L*-ornithine (40-43). GPRC6A has been found in many tissues, but the expression levels of GPRC6A in animal jejunum and colon are the highest (44). In the intestinal GLUTag cell line, GPRC6A was activated by *L*-ornithine and able to regulates hormone (e.g. GLP-1) secretion (45). The activation of GPRC6A was potentiated by divalent cations including calcium and magnesium, in physiologically relevant concentrations (43, 46), suggesting a direct role for GPRC6A in *L*-amino acids-triggered hormone secretion. However, GPRC6A was hardly detectable in FACS-sorted intestinal EECs, which raised the question that whether GPRC6A is involved in amino acid-triggered hormone secretion in primary intestinal L cells.

4.3. Sodium-dependent neutral amino acid transporter 2

Sodium-dependent neutral amino acid transporter 2 (SNAT2), the ubiquitous member of SLC38 family, participates in transmembrane transport of small neutral amino acids (47, 48). In competitive inhibition test, SNAT2 shows high affinity to alanine, proline, methionine and serine, but not charged amino acids (e.g. glutamate and lysine) and large amino acids (e.g. leucine, valine and phenylalanine) (49). In primary L cells, SNAT2 senses glutamine and elevates intracellular concentration of calcium, triggering GLP-1 secretion (50).

5. SIGNALING PATHWAYS IN AMINO ACID SENSING

5.1. Phosphatidylinositol signaling pathway

In phosphatidylinositol signaling pathway, amino acids bind to membrane receptors to activate PLC and cleave the phosphatidylinositol-(4,5)-bisphosphate (PIP2) into two second messengers, IP3 and diacylglycerol (DAG) (51). The increase of IP3 leads to the open of IP3-gated calcium channel on the membrane of intracellular calcium pool. At the meantime, the rise of calcium ions activates the transient receptor potential cation channel subfamily M member 5 (TRPM5) to promote membrane depolarization (52). DAG activates PKC and protein kinase D (PKD), which turn off the K^+ channel by phosphorylation, leading to membrane depolarization of L cell and gut hormone secretion (53).

5.2. cAMP pathway

In cAMP pathway, amino acids activate α-gustducin (Gg) after binding to membrane receptors in L cells. The activated Gg causes the increase of cAMP by activating intracellular adenylate cyclase (54). Next, cAMP activates cAMP-dependent PKA, resulting in phosphorylation and shut off of potassium channel. Inhibition of potassium efflux triggers membrane depolarization and turns on L type voltage-dependent calcium channels (55). Extracellular calcium influx leads to the increase of intracellular free calcium, which causes gut hormone secretion.

6. ELECTRICAL ACTIVITY IN AMINO ACID SENSING

L cells has electrical excitability and direct reactivity to amino acids (50, 56). When amino acid is sensed by L cells, membrane depolarization and electrical activity could be triggered, activating the influx of calcium through L or N type voltage-gated calcium channel (57-59). Patch clamp is a classical electrophysiological technique for the study of electrical activity in amino acid sensing (9). We recently recorded glutamine-triggered electrical activity of L cells by using microelectrode array. Figure 2 displayed the recorded signals of STC-1 cells. The representative 8 channel signals with negative peaks were potentials triggered by glutamine. These potentials were recorded with amplitudes about 300-500 μV in our study. Also, the signals were similar in these channels, indicating the synchronized activities in cell networks.

6.1. Patch clamp

Patch clamp was firstly introduced by Erwin Neher and Bert Sakmann on the basis of double-electrode voltage clamp in the 1970s (60). Since the 1980s, patch clamp has been widely used in the studies of ion channels, membrane proteins and cellular signaling pathways (61). Patch clamp, especially perforated-patch and standard whole-cell patch clamp recordings, allows scientists to record electrical activity of a single cell, providing a direct way for understanding the electrophysiological characteristics of L cells.

In the GLUTag model, amino acids have been shown to cause membrane depolarization, electrical activity and influx of Ca^{2+} by using patch clamp technology (62, 63). Action potentials were fired by amino acids and maintained by depolarizing current injections. Moreover, voltage-gated Na^+ channels may

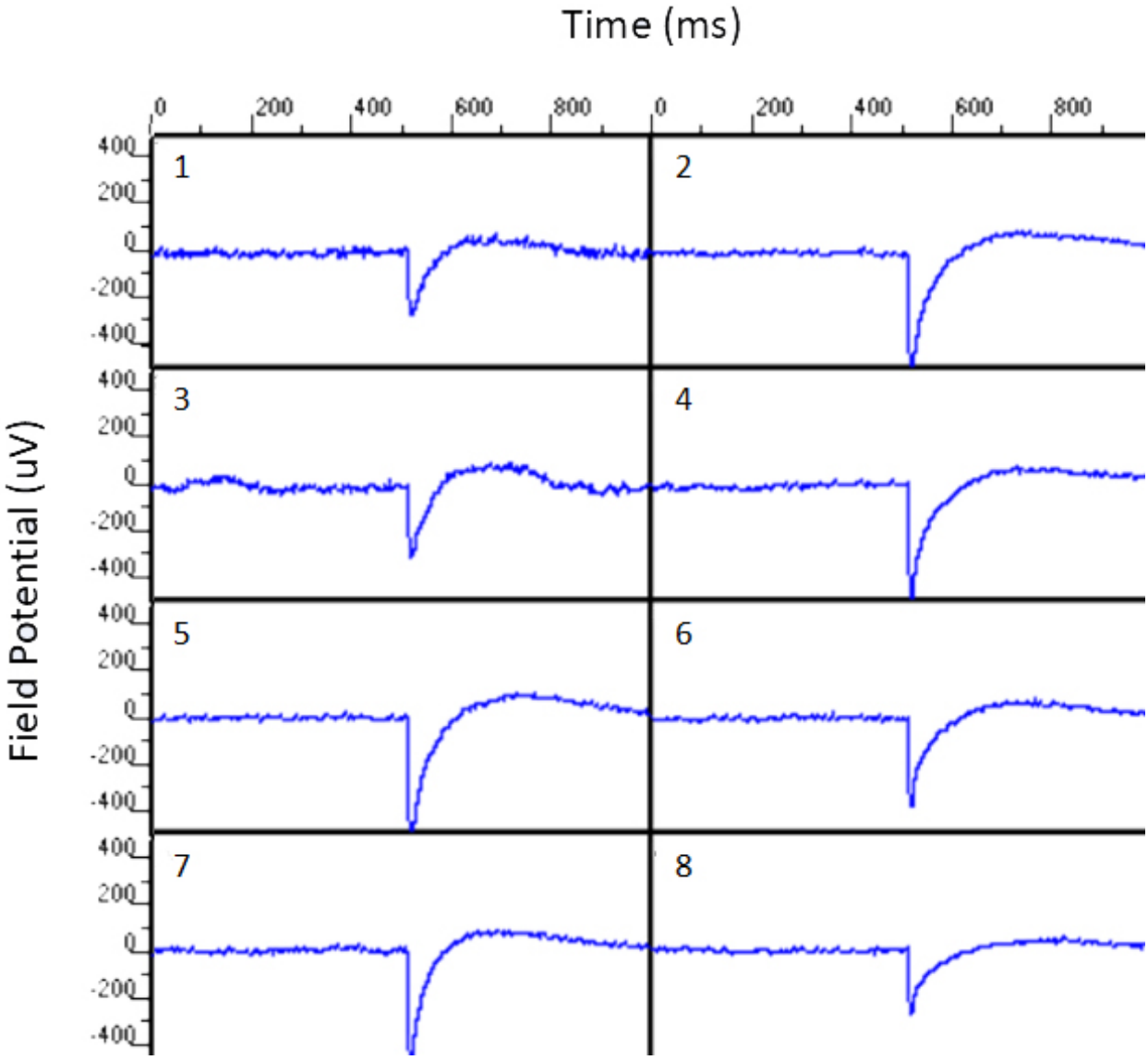

Figure 2. Glutamine-triggered electrical signals recorded by microelectrode array

also play a significant role in amino acid-triggered hormone secretion. Glutamine-stimulated GLP-1 secretion was obviously inhibited by TTX. Alanine has been proved to activate glycine receptor while asparagine and glutamine depolarize the cells by their Na^+-coupled electrogenic uptake (64, 65). Although amino acid-induced action potentials were observed in GLUTag cells, functional linkage between electrical activity and secretion was not fully established (66).

The electrical activity of primary L cells is similar to that of GLUTag cell line. Na^+ channel-dependent action potentials were fired by amino acids and the glutamine-stimulated secretion was TTX-sensitive, pointing out that GLP-1 release from primary L cells is dependent on Na^+ channels (67, 68). Na^+-dependent action potentials convert localized signals to a frequency-encoded message that can travel large distances. Therefore, it is speculated that L cells might transfer information from a portion of the cells to another via action potentials.

6.2. Microelectrode array

Microelectrode, also known as ultramicroelectrode, is an electrode smaller than 100 μm. Microelectrode array (MEA) is collector electrode in combination of multiple single microelectrodes. Culturing tissues or cells onto MEA chip, it can simultaneously record extracellular electrical activities collected from spatially distributed microelectrodes in real time (a typical MEA chip is shown in Figure 3). MEA is based on microwire arrays developed in the 1950s (69). In 1972, Thomas and colleagues found

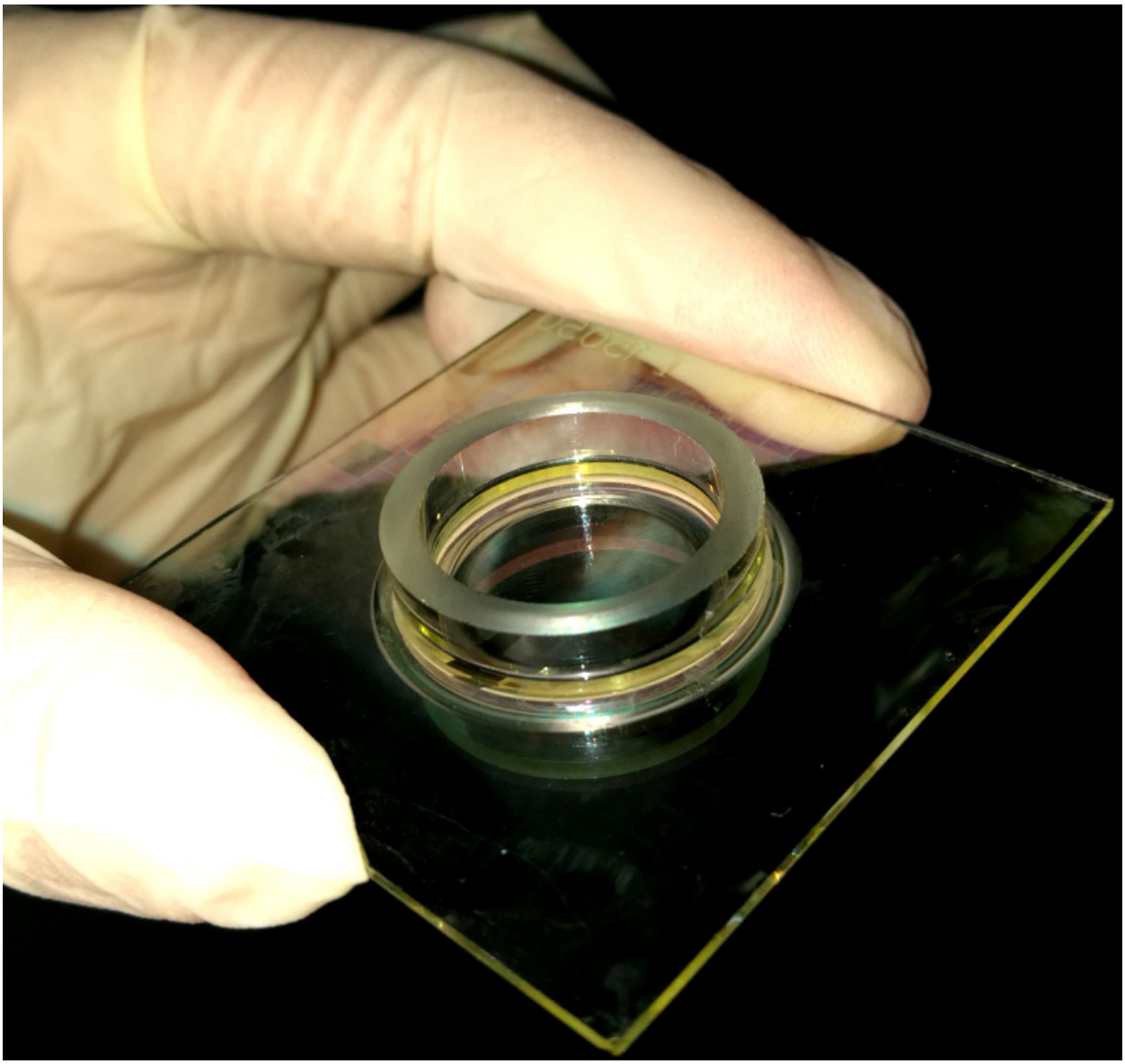

Figure 3. Microelectrode array.

that electrical activity can be recorded extracellularly with MEA (70). Over the past decades, many peer-reviewed articles have proven that the MEA technology is indeed a powerful tool in electrophysiology research and the technology around the MEA has improved significantly (71-76). Compared to ordinary electrodes, MEAs have lower signal-to-noise ratio and higher measurement sensitivity. This non-invasive electrophysiological technique is superior to the patch clamp method. MEA can be applied to a wide variety of cells, especially in the study of excitable cells such as neurons (77), cardiomyocytes (78), muscle fibers (79), and pancreatic beta cells (80). Recently, we found MEA is also a powerful tool in study of amino acid sensing. We developed a system for extracellular electrical activity monitoring based on MEA that utilizes a two-dimensional confluent layer of STC-1 cell line. STC-1 cells could be cultured onto specific MEA chip. Using a special software, we recorded strong electrical signal from STC-1 cells that triggered by glutamine. Waveform, amplitude and frequency of the electrical signals were varied in different conditions. By analyzing these parameters, we characterized the electrical process of glutamine sensing in STC-1 cells (Ding *et al.*, manuscript in preparation).

7. REGULATION OF GUT HORMONE SECRETION

Amino acids regulate secretion of various hormones such as GLP-1, GLP-2 and PYY in L cells by activating specific signaling pathways that trigger

cellular electrical activities (81-83). These hormones work independently or collaboratively with each other to regulate appetite and maintain energy homeostasis (35).

7.1. *L*-glutamine

L-glutamine can stimulate GLP-1 secretion in primary L cells and GLUTag cell line (58, 84, 85), which is related to the increase of cell excitability and change of cAMP concentration. Slc6a19 (B0AT1), a sodium-dependent transporter, has much higher expression levels in L cells than other adjacent cells (86), and may play an important role in glutamine sensing. Moreover, cAMP is also important in glutamine-triggered hormone regulation (56). In GLUTag cell line, glutamine increases concentration of cAMP and triggers ion channels open, membrane depolarization and hormone secretion (87). We showed that glutamine can induce a dose-dependent regulation of GLP-1 secretion accompanying with electrical signals (Figure 3).

7.2. *L*-arginine

L-arginine is an insulin secretagogue that stimulates GLP-1 secretion from isolated rat intestine. It has been shown that the levels of GLP-1 and insulin were increased in plasma of normal and diet-induced obese mice following the intragastric administration of *L*-arginine but not in GLP-1 receptor knockout mice (88) indicating that *L*-arginine acts as GLP-1 agonist *in vivo*. Nevertheless, *L*-arginine fails to stimulate GLP-1 secretion in GLUTag cell line *in vitro*, which raises the question that the regulation of hormone secretion by *L*-arginine relies on intact intestinal environment (58).

8. L CELLS AND HUMAN DISEASES

As L cells can regulate appetite, nutrient absorption and energy homeostasis by secreting various hormones, dysfunction of L cells can lead to disease phenotypes, for example, type 2 diabetes (89). Occurrence of type 2 diabetes is closely related to insufficient secretion of GLP-1 from L cells after meal (90). Diabetic patients have much higher blood sugar level and lower GLP-1 level than healthy controls after eating high-calorie food (91). GLP-1 and its analogues can reduce blood sugar and glycosylated hemoglobin, enhance insulin sensitivity, reduce fatty tissues and improve symptoms of type 2 diabetes (92). Hypodermic or intravenous injection of GLP-1 significantly decreases blood sugar of diabetic patients both before and after meal (93).

Obesity is also related to L cells. GLP-1 secreted from L cells can bind to thalamic nuclear receptors in hypothalamus and subsequently-induce satiety and reduce appetite (94). In addition, PYY also participates in the regulation of food intake. Injection of PYY reduces appetite and weight gain (95). Interestingly, elevated PYY in the body has been considered as one of the mechanisms that acupuncture treatment for weight loss (96).

Other diseases such as irritable bowel syndrome (97), acute pancreatitis (98), colon cancer (99) and breast cancer (100) also have been linked with L cell and hormones secretion.

9. FUTURE PERSPECTIVES

Although, many receptors and signaling pathways have been proved to play important roles in amino acid sensing, little is known about the interaction and synergistic action of these receptors. Thus, more research on signal transduction network of various intestinal amino acid receptors are warranted. Amino acid sensing in L cells triggers cellular electrical activities, these electrical activities are closely related to gut hormone secretion. However, the accurate relationship between electrical activities and hormone secretion remains unclear. A deeper understanding of the mechanisms of electrical activities in amino acid-triggered hormone secretion is important. Additionally, more transdisciplinary technologies are needed to investigate the mechanisms of amino acid sensing in the future. For example, it is anticipated that molecular biology methods combine with microscopic imaging and electroanalytical chemistry techniques would open a new horizon in amino acid sensing research.

10. ACKNOWLEDGEMENT

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