Regulation and Mechanism of Phosphatidylinositol Signaling Pathway in Animal Feed Intake: Postprint

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Date: 2017-10-10T00:00:00+00:00

Abstract

The phosphatidylinositol signaling pathway refers to the process whereby extracellular signals, upon binding to membrane receptors with their corresponding first messenger molecules, activate membrane-bound Gq protein (a type of G protein), which in turn activates phospholipase C (PLC) on the plasma membrane; PLC hydrolyzes phosphatidylinositol-4,5-bisphosphate to generate two second messengers, inositol trisphosphate and diacylglycerol. The role of this pathway in animal feeding is primarily mediated through various nutrients stimulating Gq protein-coupled receptors to activate the entire pathway. This review summarizes the structure of the phosphatidylinositol signaling pathway, its regulatory effects on animal feeding, and the underlying mechanisms, aiming to provide a reference for related research.

Full Text

The Phosphatidylinositol Signaling Pathway' s Regulation of Animal Feed Intake and Its Mechanism

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Abstract: The phosphatidylinositol signaling pathway is a process in which extracellular signals bind to their corresponding first messenger molecules via membrane receptors, thereby activating Gq proteins on the membrane and stimulating phospholipase C (PLC) on the plasma membrane. PLC then hydrolyzes phosphatidylinositol-4,5-bisphosphate to generate two second messengers: inositol trisphosphate (IP3) and diacylglycerol (DAG). The role of this pathway

in regulating animal feed intake is primarily accomplished through the activation of the entire pathway by various nutrients stimulating Gq protein-coupled receptors. This review summarizes the structure of the phosphatidylinositol signaling pathway, its regulatory effects on animal feed intake, and the underlying mechanisms, aiming to provide a reference for related research.

Keywords: phosphatidylinositol pathway; Gq protein-coupled receptor; mechanism; feed intake regulation

The phosphatidylinositol signaling pathway (phosphatidylinositol signal pathway) refers to the process where extracellular signals bind to their corresponding first messenger molecules via membrane receptors, activating Gq protein-coupled receptors (GqPCRs) on the membrane. The activated α subunit of the Gq protein dissociates from the β and γ subunits, and phospholipase C (PLC) on the plasma membrane is activated by the energy released from GTP hydrolysis. PLC then hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) to generate inositol trisphosphate (IP3) and diacylglycerol (DAG), two second messengers. Consequently, the phosphatidylinositol signaling pathway is also known as the "dual messenger system." As important signaling molecules in cells, IP3 regulates calcium ion release, while DAG modulates protein kinase C (PKC) activity, thereby controlling cellular functions such as proliferation, differentiation, contraction, secretion, and metabolism. Numerous studies have demonstrated that the vast majority of peptide hormones exert their effects through this pathway.

1 Structure of the Phosphatidylinositol Signaling Pathway

The phosphatidylinositol signaling pathway primarily comprises G protein-coupled receptors that receive stimuli, G proteins that transduce signals, PLC on the plasma membrane, PIP2 that is hydrolyzed, and the "dual messengers" IP3 and DAG. This review focuses on the initiation of the pathway—G protein-coupled receptors—and the primary functional effectors of the pathway, the "dual messengers," without detailed discussion of other components.

1.1 G Protein-Coupled Receptors

G protein-coupled receptors constitute a large family of membrane protein receptors. A schematic diagram is shown in Figure 1 [Figure 1: see original paper]. Various ligands such as odors, pheromones, hormones, neurotransmitters, and chemokines can bind to G protein-coupled receptors. Upon ligand binding, the receptor undergoes conformational changes, exhibiting guanine nucleotide exchange factor (GEF) characteristics by exchanging GDP for GTP on the G protein, thereby dissociating the α subunit from the β and γ subunits and activating the G protein for subsequent physiological processes. In the phosphatidylinositol signaling pathway, Gq protein-coupled receptors receive external stimuli. Gq protein-coupled receptors exist in diverse forms, with GPR120 being one example. Gq protein-coupled receptors are present throughout the

bodies of various animals and humans, such as $ $2-adrenergic receptors. Gq protein-coupled receptors mediate multiple signaling pathways, including the phosphatidylinositol signaling pathway, which is therefore present across various animal species. Extensive research indicates that both G protein-coupled receptors and the multiple second messengers generated by downstream pathways exert regulatory effects on T-type calcium channels, which in turn regulate feed intake by modulating calcium levels—a mechanism detailed below. Additionally, GqPCRs can activate the entire pathway through various receptor forms, thereby regulating glycolysis and hepatic glycogen metabolism.

1.2 Structure of IP3

IP3 induces the release of calcium ions (Ca2+) from intracellular calcium storage sites, thereby rapidly increasing cytosolic Ca2+ concentration. The structure of IP3 is shown in Figure 2 [Figure 2: see original paper]. IP3 is water-soluble and can diffuse throughout the cell upon generation. IP3 binds to IP3-gated calcium channels, activating these channels and elevating intracellular Ca2+ concentration, which subsequently activates various calcium-dependent proteins. IP3 signaling is terminated through dephosphorylation to form inositol bisphosphate (IP2) or phosphorylation to form inositol tetrakisphosphate (IP4). Cytosolic Ca2+ can be expelled from the cell via calcium pumps on the plasma membrane or Na+-Ca2+ exchange channels, or resequestered into the endoplasmic reticulum by calcium pumps on the ER membrane. Studies have shown that Ca2+ can regulate lipid anabolism through the IP3 pathway. Chen et al. demonstrated that reduced Ca2+ levels affect insulin secretion.

1.3 Structure of DAG

DAG is an ester formed by the condensation of two hydroxyl groups on a glycerol molecule with two fatty acids, resulting in the loss of two water molecules. DAG associates with the plasma membrane and can activate membrane-bound PKC. PKC resides in the cytosol in an inactive form under normal conditions. When G protein-coupled receptors on the cell surface receive stimuli and ultimately generate IP3, the resulting increase in Ca2+ concentration causes PKC to translocate to the inner surface of the plasma membrane, where it is activated by DAG. PKC can phosphorylate serine/threonine residues on proteins, eliciting diverse responses in different cells, such as secretion, differentiation, and muscle contraction. Montell et al. reported that DAG accumulation can desensitize insulin-stimulated glucose uptake in muscle tissue.

2 Effects and Mechanisms of the Phosphatidylinositol Signaling Pathway on Animal Feed Intake

A schematic diagram of the phosphatidylinositol signaling pathway is shown in Figure 3 [Figure 3: see original paper]. Feed intake is a crucial indicator for evaluating animal health and product quality, representing a significant fac-

tor affecting production efficiency. Early research reported that animal feed intake regulation is associated with gastrointestinal functional status (gastrointestinal motility and emptying), hypothalamic neurohumoral regulation, and peripheral humoral signals (gastrointestinal hormones, cytokines, cellular hormones, etc.). Feed intake regulation can be divided into peripheral and central mechanisms. Peripheral regulation primarily involves hormones produced in the stomach and intestine, such as insulin, while central regulation involves the coordinated action of neurons and neurotransmitters. The orexigenic and anorexigenic neurons in the hypothalamic arcuate nucleus constitute critical sites for feed intake regulation. Numerous nutrients can regulate feed intake by activating the phosphatidylinositol signaling pathway. For example, insulin release is regulated through this pathway by increasing free Ca2+ concentration, thereby modulating glucagon-like peptide-1 production and insulin secretion. Similarly, long-chain fatty acids induce cholecystokinin secretion via the phosphatidylinositol signaling pathway. Below, we describe three mechanisms and their effects on animal feed intake.

2.1 Calcium' s Effect on Animal Feed Intake via the Phosphatidylinositol Signaling Pathway

Calcium, as a mineral element, serves multiple functions, and calcium deficiency in adolescents can lead to picky eating. Calcium can be added as a mineral element to activate the phosphatidylinositol signaling pathway, thereby regulating feed intake. Lü et al. fed mice a high-fat diet for 30 days to establish an obesity model, then divided obese mice into control, norepinephrine (NE), heparin, calcium, calcium+NE, and calcium+heparin groups. Mice in drug-treated groups received intraperitoneal injections of 0.2 mL/day of either 0.1 mg/mL NE or 1.5 mg/mL heparin, while control and calcium groups received equal volumes of saline. The researchers measured triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations, collected adipose tissue to determine epididymal and perirenal fat mass, and analyzed calcium content in adipose tissue and liver. Using RT-PCR, they examined mRNA expression levels of transcription factors closely associated with adipogenesis (PPARγ, C/EBPα), lipogenic genes (FAS), and lipolytic genes (HSL) to investigate how calcium signals affect lipid metabolism in obese mice via the IP3 pathway. The results demonstrated that increased calcium intake inhibited IP3 receptor (IP3R) mRNA expression, thereby influencing feed intake. As shown in the mechanism diagram (Figure 3), calcium reduces IP3 levels by inhibiting IP3 mRNA expression. Decreased IP3 content leads to reduced intracellular Ca2+ concentration, which affects the intracellular Ca2+ signaling system and consequently influences various physiological processes regulated by Ca2+-dependent enzyme activities.

Furthermore, reduced Ca2+ concentration can inhibit the activation of phosphatidylinositol-4-kinase (PI4K) by Ca2+-sensitive recombinant human neuronal calcium sensor-1 (NCS-1) in neurons, preventing PI4K from phospho-

rylating phosphatidylinositol to phosphatidylinositol-4-phosphate (PI4P) and thereby affecting PIP2 resynthesis. This inhibits the entire phosphatidylinositol signaling pathway, leading to decreased free Ca2+ concentration, which weakens PPARγ and lipogenic gene FAS mRNA expression while enhancing lipolytic gene HSL mRNA expression, ultimately reducing fat accumulation. Reduced fat accumulation subsequently affects the feed intake regulation center, which adjusts feed intake by modulating insulin synthesis/secretion or leptin concentration to maintain energy reserves. Thus, calcium can regulate feed intake through the phosphatidylinositol signaling pathway.

The study by Lü et al. demonstrates that calcium regulation of animal feed intake involves three components: calcium' s modulation of the phosphatidylinositol signaling pathway, the pathway' s regulation of lipid metabolism via Ca2+ concentration, and the ultimate effect of lipid metabolism on feed intake. While this research used rodents, Wei Xingcan investigated Xinxinmei Large White pigs and found that the phosphatidylinositol signaling pathway participates in porcine lipolysis, associated with peptides derived from chromogranin A. However, whether dietary calcium supplementation affects feed intake through this pathway in Large White pigs requires further investigation.

2.2 Orexin's Effect on Animal Feed Intake via the Phosphatidylinositol Signaling Pathway

Orexin is a hypothalamic neuropeptide that regulates energy metabolism, feeding, and sleep-wake cycles. It has two Gq-coupled receptors: OX1R and OX2R. Orexin neuronal cell bodies are primarily located in the lateral hypothalamic area and perifornical nucleus. Studies suggest the fourth ventricle of the hypothalamus may be one of its target sites. Orexin primarily increases feed intake and controls energy expenditure, correlating with fat accumulation. The distribution of orexin and its receptors varies among species: orexin is present in the gastrointestinal nervous systems of pigs, newborn dogs, and horses, while OX1R is found in the submucosal and muscular layers of the intestinal tract in ruminants. Dyer et al. intramuscularly injected synthetic porcine orexin at 3 mg/kg body weight into weaned piglets, observing increased food consumption after 12 hours, significantly higher cumulative feed intake between 12-24 hours, and an 18% increase in cumulative feed intake compared to controls after 24 hours, demonstrating orexin' s ability to upregulate porcine feed intake. In ruminants, Kuhla et al. reported that orexin A (OXA) functions as an appetite-regulating substance prepartum, with expression levels significantly increasing under feeding conditions. They also noted synchronous increases in PPARγ and OXA expression during fasting, significantly different from ad libitum feeding. These findings indicate that orexin regulates feed intake across various animal species.

Orexin modulates animal feeding through multiple pathways, including regulation via the phosphatidylinositol signaling pathway. Gorojankina et al. investigated OXA' s effects on Wistar rat Odora cells and found that OXA treatment

significantly increased IP3 and Ca2+ concentrations, indicating the involvement of the phosphatidylinositol signaling pathway in orexin-mediated feed intake regulation. OXA-induced Ca2+ elevation likely results from calcium channel opening. The orexigenic effect of orexin can be completely blocked by PKC-specific inhibitors, suggesting that orexin may activate the phosphatidylinositol signaling pathway by binding to Gq-coupled receptors, thereby activating PKC, opening Ca2+ channels, and increasing Ca2+ influx (Figure 3). Thus, orexin regulates feed intake through the phosphatidylinositol signaling pathway.

2.3 Ghrelin' s Effect on Animal Feed Intake via the Phosphatidylinositol Signaling Pathway

Ghrelin is an appetite-stimulating hormone produced by P/D1 cells in the gastric fundus and pancreatic base of humans, serving as an endogenous ligand for growth hormone secretagogue receptor-1A (GHSR-1A). Ghrelin is widely distributed in both central and peripheral tissues. Numerous studies have confirmed that ghrelin injection stimulates appetite and increases feed intake in humans and animals. Intravenous ghrelin administration in weaned piglets increased feeding frequency and body weight compared to saline-injected controls after 5 days. However, in poultry, intracerebroventricular ghrelin injection in chicks decreased feed intake while increasing plasma cortisol and adrenocorticotropic hormone concentrations, inducing anorexia in a dose-dependent manner. Similar effects were observed in Japanese quail. These results demonstrate species-specific differences in ghrelin' s feed intake regulation, though ghrelin indeed modulates feed intake across various animals.

Ghrelin' s regulation of feeding occurs through multiple pathways. Its growth hormone-releasing effect is mediated by GHSR-1A, which has been identified as a Gq protein-coupled receptor that exerts biological effects through PLC or adenylate cyclase (AC) upon activation. Ghrelin binding to GHSR-1A activates PLC, hydrolyzing PIP2 to produce IP3 and DAG. IP3 promotes Ca2+ release (Figure 3). Darko et al. discovered that ghrelin' s appetite control is mediated through the mTORC1/S6K1 pathway, which is activated by increased Ca2+ concentration following phosphatidylinositol signaling pathway activation. Elevated cytosolic Ca2+ levels generate calcium currents that activate AMP-activated protein kinase (AMPK) without energy consumption. AMPK phosphorylation enhances SIRT1 and its downstream FOXO1 activity while suppressing mTOR activity, thereby activating orexigenic NPY/AgRP neurons or inhibiting anorexigenic POMC/CART neurons to promote feed intake. Meanwhile, DAG acts on PKC to activate Ca2+ channels in adenohypophyseal cells, stimulating growth hormone secretion and increasing gastric emptying rate, thereby promoting feed intake. Intravenous ghrelin injection in rats (0.8-2.0 g/kg) induced dose-dependent increases in gastric acid secretion, gastric contraction frequency and amplitude, and accelerated gastric emptying.

Feed intake is a critical factor for optimal animal production performance and directly affects production efficiency. In summary, the phosphatidylinositol signal-

ing pathway mediates the regulation of feed intake by various substances across animal species, and these substances exist in the vast majority of mammals, indicating that this pathway functions in all animal types, including rodents, large mammals, and small mammals. The key to feed intake regulation by the phosphatidylinositol signaling pathway lies in the activation of Gq-coupled receptors, with its effects primarily depending on increased Ca2+ concentration and modulation of products such as PKC. Nutrient regulation of feed intake is mainly achieved through activation of the entire pathway. The phosphatidylinositol signaling pathway plays significant roles in multiple physiological processes, particularly feed intake, yet our understanding remains incomplete. Current research predominantly focuses on rodents, with fewer studies on mammals, and most investigations are confined to the medical field. This review aims to provide references for future research by introducing the phosphatidylinositol signaling pathway. With Lefkowitz and Kobilka' s Nobel Prize-winning research on G protein-coupled receptors garnering increasing attention, we anticipate that the phosphatidylinositol signaling pathway will receive greater focus and yield promising results in mammalian studies.

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