

## Review

# The PACAP/PAC1 receptor system and feeding

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**Abstract:** Pituitary adenylyl cyclase activating polypeptide (PACAP) belongs to the vasoactive intestinal polypeptide (VIP)/secretin/glucagon superfamily. PACAP is present in two forms, PACAP-38 and PACAP-27, and binds to three guanine-regulatory (G) protein-coupled receptors (PAC1, VPAC1, and VPAC2). PACAP is expressed in the central and peripheral nervous systems with high PACAP levels found in the hypothalamus, a brain region involved in feeding and energy homeostasis. PAC1 receptors are high-affinity and PACAP-selective receptors, while VPAC1 and VPAC2 receptors show a comparable affinity to PACAP and VIP. PACAP and its receptors are expressed in the central and peripheral nervous systems, with moderate to high expression in the hypothalamus, amygdala, and other limbic structures. Consistent with their expression, PACAP is involved in several physiological responses and pathological states. A growing body of literature suggests that PACAP regulates food intake in laboratory animals. However, there is no comprehensive review of the literature on this topic. Thus, the purpose of this article is to review the literature regarding the role of PACAP and its receptors in food intake regulation and to synthesize how PACAP exerts its anorexic effects in different brain regions. To achieve this goal, we searched PubMed and reviewed 68 articles regarding the regulatory action of PACAP on food intake. Here, we present the literature regarding the effect of exogenous PACAP on feeding and the role of endogenous PACAP in this process. We also provide evidence regarding the effect of PACAP on the homeostatic and hedonic aspects of food intake, the neuroanatomical sites where PACAP exerts its regulatory action, which PACAP receptors may be involved, and the role of various signaling pathways and neurotransmitters in hypophagic effects of PACAP.

**Keywords:** PACAP, PAC1, Homeostatic, Hedonic, Food Intake, Signaling, Neuroanatomical Site

## 1. Introduction

The Food consumption is necessary for survival and is affected by homeostatic and hedonic aspects of feeding. Hunger and satiety are two physiological responses that promote and reduce appetite and food intake, respectively. Signals that facilitate or impede appetite and feeding are integrated into the nucleus tractus solitarius and hypothalamus. Three hypothalamic structures play essential roles in this process. These include the ventromedial nucleus of the hypothalamus (VMN), serving to suppress appetite and food intake, the lateral hypothalamus (LH), functioning to promote appetite and food consumption, and the arcuate nucleus of the hypothalamus (ARC), operating as a switchboard to turn on or turn off the above structures to regulate appetite and food intake [1; 2]. PACAP mediates food intake in these structures in distinctive manners. For example, injection of PACAP in the VMN has shown to decrease food intake, whereas PACAP injection in the ARC shows selective decreases in carbohydrate intake but not high-fat intake. The LH's role in homeostatic feeding and inhibitory afferent projections from the BNST, shown to attenuate food intake upon PACAP injection, suggests that

PACAP may also have indirect effects on the LH's satiety dependent food intake (for detail, see Sections 4 and 8).

In the ARC, two groups of neurons exist that integrate and modulate feeding behaviors. These neurons contain anorexigenic peptides, such as proopiomelanocortin (POMC) and cocaine-amphetamine-regulated transcript (CART), as well as orexigenic peptides, such as neuropeptide Y (NPY)/agouti-related peptide (AgRP). Many central and peripheral inputs can alter the activity of these neurons. For example, insulin, leptin, ghrelin, etc., secreted by peripheral tissues, can activate one or the other type of ARC neurons to increase or decrease appetite and food intake. Changes in the levels of nutrients in blood circulation can also relay excitatory or inhibitory signals to these neurons to stimulate or suppress appetite. For example, low blood glucose increases ghrelin secretion, which increases calcium levels in NPY-containing neurons [3]. The increase in calcium facilitates exocytosis and triggers the release of NPY, promoting appetite and food intake.

Several peptides in the peripheral or central nervous system can regulate appetite and food intake. Glucagon superfamily promotes hypophagia, a conserved response throughout different species [4]. A growing body of evidence shows that pituitary adenylyl cyclase activating polypeptide (PACAP), a peptide within this superfamily, regulates food intake, suggesting that PACAP, like other analogs in the glucagon superfamily that have already been used in a clinical setting, may prove to be a potential target to develop medications to treat obesity. Likewise, PACAP's dual role in both satiety and hedonic feeding suggests that it may prove useful in treating binge-eating disorders. Thus, we reviewed the literature (68 articles selected and included in this review) regarding the regulatory action of PACAP on food intake (Table 1). In this review, we attempted to describe the literature on the role of endogenous PACAP and the effect of exogenous PACAP on food intake. Furthermore, we tried to provide information regarding neuroanatomical sites where PACAP exerts its hypophagic effects in the brain. We also discussed the effect of PACAP on the hedonic and homeostatic aspects of feeding. Finally, we reviewed the role of neurotransmitters and signaling mechanisms in the hypophagic actions of PACAP. PACAP is also involved in glucose homeostasis ([5; 6; 7; 8], for a review see, [9]). However, it is not the scope of this article to review the literature regarding the role of PACAP in glucose homeostasis and insulin secretion.

## 2. PACAP, its receptors, and its physiological roles

Pituitary adenylyl cyclase-activating polypeptide (PACAP) was initially isolated from the ovine hypothalamus as an activator of adenylyl cyclase in pituitary cells [10]. PACAP is made up of 38 amino-acid residues (PACAP38) and is C-terminally alpha-amidated; the sequence encompasses an internal cleavage-amidation site that generates a 27-residue alpha-amidated polypeptide fragment or PACAP27, which corresponds to the N-terminal 27 amino-acid portion of the PACAP38 [11].

Human PACAP27 shares 68% identity with VIP, classifying PACAP as a member of the VIP/secretin/growth hormone/glucagon superfamily. The PACAP gene contains five exons, and PACAP is encoded by exon 5. The human PACAP promoter possesses two cAMP response-like elements, which play a role in the tissue-specific factor growth hormone and six binding domains for the thyroid-specific transcription factor-1.

PACAP binds to three G protein-coupled receptors: PAC1R, VPAC1, and VPAC2 [12]. The PACAP-preferring PAC1 receptors isoforms can be coupled to  $G_s$  and  $G_q$  to stimulate adenylyl cyclase and phospholipase C (PLC), respectively. In contrast, VPAC1 and VPAC2 receptors, which had equal affinity for PACAP and VIP, are principally coupled to  $G_s$  to activate adenylyl cyclase and increase intracellular 5'-cyclic adenosine

monophosphate (cAMP) levels [13]. The rise in cAMP activates protein kinase A (PKA). PLC causes the breakdown of phosphatidylinositol and the production of inositol triphosphate and diacylglycerol, leading to an increase in intracellular calcium and protein kinase C activation. These biochemical changes modulate multiple ionic currents and stimulate signaling factors. Moreover, PAC1 receptor activation led to  $\beta$ -arrestin-mediated receptor internalization and endosomal signaling, which led to extracellular regulated protein kinase phosphorylation [14; 15].

PACAP is expressed in the central nervous system and peripheral organs and was shown to exert multiple effects (pleiotropic). These include control of neurotransmitter release, vasodilation, bronchodilation, activation of intestinal motility, increase in insulin and histamine secretion, immune modulation, and cell proliferation and differentiation [11]. A growing body of the literature also suggests that PACAP and its receptors are involved in homeostatic and hedonic aspects of feeding. PACAP exerts its anorexigenic effect in different brain regions and periphery involving insulin regulation and other mechanisms. This article aims to evaluate the literature regarding the regulatory actions of PACAP on feeding.

### 3. PACAP and Feeding

Initial studies showed that central PACAP administration reduces feeding. Morley and colleagues found that ICV injection of PACAP at 2.5 (but not 1)  $\mu$ g reduced food intake in mice [16]. Likewise, a dose-dependent decrease in food intake was reported following ICV injection of PACAP in male Sprague-Dawley rats [17]. Subsequent studies confirmed these results showing that intracerebroventricular (ICV) administration of PACAP to food-deprived male CD1 mice dose-dependently reduced food consumption. Matsuda and colleagues [18; 19] found that ICV injection of PACAP or VIP (11 and 22 pmol/g) reduced food consumption in goldfish, with the higher dose of PACAP and VIP inducing increases in locomotor activity as well. In another study using Zebrafish, ICV injection of a low dose of PACAP (2pmol/g) suppressed food intake, suggesting that PACAP acts as an anorexigenic peptide in the brain of fishes [12]. The hypophagic effect of PACAP was reduced in the presence of a PACAP receptor antagonist [20] or PACAP receptor knockout mice [21]. Additional research showed that the peptide levels were elevated in the brain stem in food-deprived rats. Similar changes occurred in the hypothalamus and telencephalon in chickens [22], suggesting that endogenous PACAP is involved in food intake regulation. In this article, we reviewed the literature regarding the role of PACAP and its receptors in food intake. We first discussed the role of endogenous PACAP in feeding. We then presented the findings regarding the effect of exogenous PACAP and related ligands on food intake. We also reviewed the literature regarding the involvement of different neurotransmitter systems and brain regions in the hypophagic effects of PACAP.

#### 3.1. The role of Endogenous PACAP in food intake

Fasting alters the level of endogenous PACAP. Jozsa and colleagues [22] reported elevated levels of PACAP in the brain stem in food-deprived rats and the hypothalamus and telencephalon in chickens, raising the possibility that endogenous PACAP may serve as a regulator of food intake. Similar results were been reported in mice. Kiss et al. (2006) found that PACAP levels were changed in mice after food and water deprivation, and there was sexual dimorphism in this regard. In the hypothalamus, brainstem, and telencephalon of male mice, PACAP levels were significantly elevated 12 h after the beginning of food deprivation, but not at 36 h and 48 h [23]. No significant differences

were observed in PACAP levels between the control and experimental conditions in the brainstem and diencephalon in female mice. In the hypothalamus of food-deprived female mice, PACAP levels showed a slight increase. When male mice were water-deprived, there was no significant change in PACAP levels, but decreases in PACAP levels in the hypothalamus of female mice were observed at the 12 and 36 h time points. The brainstem of water-deprived male mice showed slight increases in PACAP levels and returned to basal levels by 48 h. PACAP levels in female mice displayed significant decreases in the brainstem 12 h after the onset of water deprivation. There were no significant changes in PACAP levels in the telencephalon of either male or female mice. However, whether these changes are due to the stress of fasting, since PACAP was reported to regulate the stress response [24; 25; 26; 27; 28; 29; 30] or these changes occur to promote satiety in food-deprived animals need to be addressed.

Nakata and colleagues showed that food intake was reduced in PACAP-deficient mice compared to their wild-type controls, showing that endogenous PACAP may facilitate eating rather than promoting satiety [21]. They also examined the underlying mechanism of this regulatory action of PACAP and discovered that NPY mRNA was reduced in PACAP-deficient mice. Given that NPY neurons are affected by glucose and other chemicals, they also studied if high carbohydrate intake would be changed in these mice. Their results revealed that carbohydrate consumption but not a high-fat diet was reduced in PACAP knockout mice than wild-type controls [21]. Together, these findings suggest that endogenous PACAP influences carbohydrate consumption via the NPY-releasing neuron in the ARC. Relatedly, PACAP, PAC1 R, and VPAC2R are expressed on NPY-containing neurons in the ARC [31].

Consistent with the above findings, Sherwood [32] found that when PACAP knockout and wild-type mice were fed either regular chow or high-fat diet for 30 weeks, there was no difference in growth, food intake, body weight, or size of perirenal fat pads between mice of the two genotypes. However, Nguyen and colleagues [11] showed that the nocturnal and daily food intake was reduced in PACAP knockouts compared to wild-type mice; however, diurnal food intake showed an increase in PACAP knockouts, suggesting that differences in food intake in mice lacking PACAP may be dependent on the time of day.

The PACAP system may also be involved in glucose homeostasis. Gray and colleagues reported that glycogen levels were reduced in mice lacking PACAP compared to their wild-type controls [33]. These researchers discovered that the fasting glucose level was lower with a concomitant increase in insulin levels in PACAP-deficient mice than their wild-type controls [33]. Furthermore, there was an increase in cholesterol, triglycerides, and free fatty acid (although the latter was insignificant) in PACAP knockout mice. These results show that the metabolism of carbohydrates and lipids is impaired in the absence of PACAP [33].

The regulatory effect of PACAP on food intake may be mediated via the melanocyte-stimulating hormones (MSH) system [34]. These authors showed that food deprivation increased the expression of NPY and reduced the expression of proopiomelanocortin (POMC), the precursor of the anorexigenic peptide  $\alpha$ -MSH. Similar changes were observed in the PACAP gene expression [34], indicating that PACAP may serve as another anorexigenic peptide. Indeed, these authors showed that exogenous PACAP administration reduced food intake in food-deprived animals. This effect of PACAP was reduced but not completely abolished by the PACAP receptor antagonist, PACAP6-38. The antagonist alone did not affect food intake. The authors concluded that PACAP acts on PAC1 receptors to reduce food intake. Although this conclusion may be plausible, considering that PACAP acts on VPAC1 and VPAC2 receptors and that PACAP6-38 has

some actions at VPAC receptors [35; 36], further studies are needed to characterize the receptor types involved in this process.

PACAP levels in the VMN of mice [37] and goldfish [18] are upregulated by chronic overeating. On the other hand, its expression is reduced by food deprivation in both the whole hypothalamus and the VMN of the mouse. Moreover, in the rat hypothalamus, PACAP protein levels are increased 12 hours after fasting but returning to basal levels by 36 h. Gargiulo and colleagues concluded that PACAP regulates food intake through the negative feedback loop under normal homeostatic feeding, this may become dysregulated under food addiction [reviewed in [38]].

Some studies revealed that mice lacking the PACAP gene showed lower food intake levels. These mice also had reduced NPY expression in the ARC, explaining the reduced high-carbohydrate consumption. Contrastingly, other studies reported that when rats were administered PACAP-38 locally in the nucleus accumbens, they showed a reduction in binge-eating and hedonically driven consumption of fats and carbohydrates [39].

Researchers proposed that chronic exposure to tasty solutions induces changes in the expression of the splice variants of the PAC1 receptor and thus changes in PAC1R binding and signaling. In areas of the brain in which both PAC1 and VPAC receptors were present, this change of the variant of PAC1 receptor may favor signaling towards either type of receptor, leading to differences in behaviors [[40]; for an excellent review, see [38]].

Burgos and colleagues measured changes in food intake and body weight induced by cocaine/amphetamine-regulated transcript (CART) in the presence of subcutaneous injection of PACAP6-38 [41]. While PACAP6-38 reduced the ability of CART to decrease food intake and body weight, it did not alter the motor stimulatory effect of CART. PACAP6-38 also had no effects by itself on feeding after peripheral administration. The authors concluded that there might be multiple CART receptors. Alternatively, CART may be acting via the release of endogenous PACAP to reduce food intake. Also, PACAP6-38 might act as an agonist on the VPAC receptors to alter the action of CART on food intake but not on locomotion.

Recently, Hannibal and colleagues [42] studied the role of PACAP in food anticipatory activity (FAA). Researchers found that when knockouts and wild-type mice entrained to both the full photoperiod (FPP) and skeleton photoperiod (SPP) were placed on a restricted feeding diet, knockout mice showed an earlier onset of FAA, greater weight loss, and increased activity than their wild-type counterparts at both photoperiod conditions at the 10-lux light intensity, but only at the SPP condition at the 300-lux light intensity [42].

### **3.2 The effect of exogenous PACAP and related ligands on food intake**

Earlier studies showed that central PACAP administration reduces feeding. Morley and colleagues found that ICV injection of PACAP at 2.5 (but not 1)  $\mu\text{g}$  reduced food intake in mice [16]. Matsuda and colleagues found that ICV injection of PACAP or VIP (11 and 22 pmol/g) reduced food consumption in goldfish, with the higher dose of PACAP and VIP inducing increases in locomotor activity as well [19]. In another study using Zebrafish, ICV injection of a low dose of PACAP (2pmol/g) suppressed food intake, suggesting that PACAP acts as an anorexigenic peptide in the brain of fishes [12]. However, certain synthetic forms of PACAP did not display hypophagic effects at a 10-fold higher dose, indicating that the peptide is anorexigenic only at low doses or the synthetic peptides may desensitize the receptor at higher doses. The study also found that locomotor activity was unchanged before and after the administration of synthetic/natural peptides, suggesting that PACAP-induced hypophagia was not a result of motor

impairments. However, we found that ICV injection of higher doses of PACAP reduced locomotor activity in C57BL/6J mice [43]. Thus, the difference could be due to different doses of the peptide vs. synthetic ligands and/or the use of different species of animals.

Preclinical studies reported that exogenous PACAP and related ligands mimic the regulatory action of endogenous PACAP on feeding. Earlier studies showed a dose-dependent decrease in food intake following ICV injection of PACAP in male Sprague-Dawley rats. The reduced food intake was observed for 16 h following ICV injection; the effect was evident also at 24 h and 48 h but returned to control level at 72 h after PACAP injection. Additionally, body weight decreased, and the rats were more energetic after PACAP administration. PACAP also increased mRNA levels of NPY in the ARC and that of galanin in the PVN. On the other hand, it decreased the level of CRH in the PVN. Rats that were deprived for 72 h expressed greater NPY mRNA compared to fed rats. CRH mRNA was lower, and galanin mRNA was not affected by fasting. Overall, these results suggest that PACAP exerts hypophagia, but this effect may be independent of changes in the expression of hypothalamic peptides involved in food intake [17].

ICV PACAP administration to food-deprived male CD1 mice dose-dependently reduced food consumption for at least 3 h. This effect of PACAP was attenuated by PACAP6-38, a PACAP receptor antagonist, but was not mimicked by VIP, suggesting that PACAP exerts its anorexigenic action through the PACAP-selective PAC1 receptors [34]. However, additional studies are needed to delineate the role of each PACAP receptor on the hypophagic effect of the peptide because PACAP6-38 was shown to possess actions on other PACAP receptors [35; 36].

Systemic PACAP administration also reduces feeding. For instance, intraperitoneal injection of PACAP38 and PACAP27 before the dark cycle in mice reduced food intake, meal duration, meal frequency, and feeding bouts. The regulatory actions of these peptides were absent in mice lacking PAC1 receptors, suggesting that PACAP reduces feeding via the PAC1 receptors [20]. These authors also showed that the regulatory actions of PACAP might be through decreases in plasma ghrelin levels, with no change in glucagon-like peptide 1 (GLP-1), glucagon, PYY, and insulin levels. Correspondingly, the ghrelin level was significantly elevated by overnight fasting and postprandially in PAC1 receptor knockout mice compared to their wild-type controls. These results suggest that PACAP may reduce food intake via inhibiting the action of ghrelin in the hypothalamus. Alternatively, PACAP may serve as a regulator of ghrelin secretion at the periphery.

Endogenous PACAP may also regulate the release of other peptides, such as insulin and leptin, involved in energy homeostasis. Indeed, these peptides' level was reduced in PAC1 knockout mice under fasting state and postprandially [20]. The level of GLP-1 was also reduced but only postprandially, whereas the level of PYY and glucagon was unchanged in PAC1 knockout mice. These findings suggest that the PACAP/PAC1 receptor system could be a potential target to develop medications to treat obesity and diabetes.

The effect of PACAP on blood glucose in Goto Kakizaki (GK) rats and C57BL/6 mice fed a regular versus high-fat diet was studied [44]. The authors found that PACAP38 (0.6 and 60 pmol/kg/day, i.p.) administration at three weeks of age till 8-10 weeks reduced blood glucose in GK rats, an animal model of type 2 diabetes. They also assessed the effect of PACAP on high-fat diet-induced type 2 diabetes in C57BL/6 mice. The results showed PACAP27 (50 pmol/kg/day for five days) reduced blood glucose in C57BL/6 mice regardless of being fed a regular or high-fat diet. These studies suggest that PACAP reduces glucose in animal models of type 2 diabetes. However, more work is needed to describe how PACAP regulates glucose homeostasis. For example, it needs to be

determined whether PACAP exerts its action on insulin secretion or other mechanisms may be involved [44].

The endogenous PACAP may also regulate blood glucose. Green and colleagues (2006) found that blood glucose levels were significantly elevated at 30 and 60 minutes after a glucose challenge in mice injected with PACAP6-27 compared with mice treated with saline. The authors concluded that acute inhibition of PACAP signaling leads to poor glucose tolerance. These authors also showed that once-daily injection of PACAP6-27 for two weeks led to impaired glucose tolerance, the exaggerated glycemic response to feeding with no change in insulin release. Plasma glucagon, triglycerides, total cholesterol, HDL levels were unchanged [45], suggesting a decrease in insulin receptor sensitivity or signaling mechanism. When insulin was administered intraperitoneally, knockout mice fed a high-fat diet but not regular chow showed decreased glucose levels compared to wild-type mice, suggesting that knockouts are more insulin-sensitive than their wild-type controls [32].

In male layer chicks, the central administration of PACAP and VIP each exerted anorexigenic effects. These effects were mediated by the corticotropin-releasing factor (CRF), as this action of the peptides was reduced by astressin, a CRH receptor antagonist [46]. However, the CRH antagonist had a differential effect on the anorexigenic effect of PACAP and VIP. Astressin significantly reversed the anorexigenic effects of PACAP at 90 minutes with lesser effect at earlier time points. In contrast, it significantly reversed the hypophagic effect of VIP throughout the 90-min test period, suggesting CRH is differentially involved in the hypophagic effect of VIP and PACAP. Both peptides also increased corticosterone secretion via the CRH system. However, VIP-induced corticosterone secretion was only partially attenuated by astressin [46].

Maxadilan, a PAC1R agonist, also reduced food intake in NIH mice [47]. However, M65, the PAC1R antagonist, did not alter food intake in these mice. These authors demonstrated that maxadilan increased food intake upon repeated administration, requiring further investigation to delineate the underlying mechanisms of acute vs. chronic effects of PACAP ligands on food intake and body weight.

The effect of systemic administration of PACAP was evaluated on food intake in rodents. For example, Vu and colleagues [20] examined the effect of PACAP on appetite and food intake. These authors injected PACAP38 or PACAP27 intraperitoneally in PAC-1 receptor-deficient mice and their wild-type controls and found that PACAP, in the periphery, can suppress appetite in a dose-dependent manner in wild-type but not in PAC1R knockout mice, showing that PACAP reduces food intake via the PAC1R in the periphery. The authors also showed that PACAP reduced ghrelin levels without changing GLP-1, PYY, insulin, and glucagon levels. Correspondingly, the ghrelin level increased to a greater extent in knockout animals than their wild-type controls regardless of whether animals were kept fasted or not. These results suggest that not only exogenous PACAP but also endogenous PACAP regulates the level of ghrelin. Interestingly, the levels of other peptides such as insulin, leptin, and GLP-1 were reduced in PAC1R (-/-) mice. However, in another study, PACAP27 (10-100 fmol) altered insulin secretion from the pancreatic beta cells, which was glucose-dependent and mediated by an increase in calcium concentration in beta cells [48]. PACAP27 stimulated insulin secretion and increased calcium concentration inside the beta cells in the presence of 8.3 mM but not 2.8 mM glucose. PACAP38 also increased the release of insulin [48]. The effect of PACAP was blocked by nitrendipine showing the involvement of calcium influx via the L-type calcium channels in insulin exocytosis. VIP also did increase insulin secretion but at a higher concentration. PACAP38 mimicked the effect of PACAP27. The authors concluded that

PACAP could act as a neuronal or local hormonal regulator of glucose-induced insulin secretion [48].

In another study, PACAP was shown to promote glucose output [49]. For example, these authors showed that PACAP27 concentration-dependently increased glucose output from the rat liver in a calcium-dependent manner. The same effect was observed with PACAP38, whereas VIP only slightly increased glucose output. Correspondingly, there was an increase in cAMP levels by PACAP27 and PACAP38. When theophylline was added to the perfusate, the cAMP output induced by 40 nM PACAP27 increased significantly; however, the degree of glucose output was not affected. On the other hand, when the effect of PACAP on glucose output and cAMP accumulation was studied in a calcium-free medium, the stimulatory effect of PACAP on glucose output was blunted without any effect on cAMP accumulation [49]. Together, these results suggest that PACAP may elevate glucose output by increasing calcium but not cAMP, suggesting that it may involve the PKC pathway but not the PKA pathway. However, further studies are needed to confirm the involvement of these pathways in the ability of PACAP to increase hepatic glucose output.

Intraperitoneal PACAP injection increased body weight (without altering hepatosomatic index) via growth hormone through protein increase and lipid mobilization during ovulation in juvenile tilapia and catfish. Injection of VIP at a relatively higher dose (88 pmol/g, i.p.) did not attenuate food consumption, indicating that VIP at a high dose may desensitize the pathways involved in food intake [50]. Matsuda and researchers (2005) found that injection of VIP (22 and 44 pmol/g, i.p.) decreased food intake, while 88 pmol/g of VIP did not have this effect. On the other hand, PACAP decreased food consumption at all doses administered (22, 44, and 88 pmol/g, i.p.).

#### **4. Neuroanatomical sites of PACAP action in regulating food intake**

Different brain regions are implicated in the regulatory actions of PACAP on the homeostatic and hedonic aspects of food intake (Figure 1). The bed nucleus of stria terminalis (BNST) is important in regulating anxiety-like behavior caused by long periods of threats and has a role in mediating stress-induced anorexia and associated reductions in weight gain [51; 52]. PACAP may act in the BNST to regulate stress response during stressful events. An earlier study [53] showed that infusion of PACAP38 in the posterior BNST in male and female Sprague-Dawley rats mimics weight loss and anorectic state induced by repeated stress exposure. But this effect was not observed following PACAP administration in the anterior BNST or ICV, showing that PACAP exerts its action specifically in the posterior BNST. The authors concluded that in female rats, the site-specific action of BNST PACAP was inconclusive. Given the role of PACAP and the importance of BNST in the regulation of food intake and stress response, the PACAP/PAC1R system may be involved in stress-related eating disorders.

In an earlier study [54], PACAP was injected directly into the VMN increased both core body temperature and locomotor activity for up to 7 h. When PACAP (25pmol) was administered locally into the VMN, feeding decreased by 52%, which lasted for 3 h, while PACAP at 50pmol reduced feeding by 80% for up to 6 h. The study also found that when PACAP was injected into the VMN, POMC mRNA expression was greater in the ARC, without any change in NPY or AgRP mRNA activity [54]. Considering that the VMN was shown to project onto the nucleus tractus solitarius (NTS), which is responsible for regulating energy homeostasis, food intake, and locomotor activity, researchers concluded that excitation of the VMN via PACAP injections might be responsible for increased POMC signaling within the NTS [54].

When PACAP was injected into the PVN or VMN, it reduced the latency in consuming the first meal and the rate of eating in Sprague-Dawley rats as compared to the vehicle. When injected in the PVN, rats showed strong decreases in meal size, meal duration, and total time spent eating. These effects were reversed by PAC1R antagonists but not VPAC antagonists. Researchers also found that PACAP administration in both the PVN and VMN increased plasma glucose concentration but did not affect pancreatic hormone levels. Upon injection of PACAP in the VMN, core body temperature and spontaneous locomotor activity in rats were elevated compared to controls. These results were reproduced using maxadilan, a PAC1R agonist, but not after VIP injections in either brain region [55].

Iemolo and colleagues found that rats infused with PACAP in the central amygdala showed decreased food intake and body weight with no significant locomotor activity changes [56]. However, intra-basolateral amygdala infusion of PACAP was without any effects on food intake and body weight or locomotor activity, highlighting the site-specificity of hypophagic action of PACAP.

### 5. The role of other neurotransmitters in the actions of PACAP on food intake

Anorexia induced by central injection of PACAP is mediated, in part, through activation of the melanocortin system, as the POMC gene expression was increased by PACAP and reduced in PAC1 receptor knockout mice. Additionally, PACAP increased *c-fos* expression in POMC neurons. Furthermore, the hypophagic effect of PACAP was reduced by SHU9119, an MC3/MC4 receptor antagonist, which had no significant effect by itself on food consumption [34]. These authors concluded that central administration of PACAP exerts its anorexic effect, at least in part, through activation of the hypothalamic melanocortin system [34].

As stated above, activation of POMC neurons suppresses food intake and appetite via the release of the  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), an endogenous melanocortin receptor agonist. In contrast, AgRP inhibits POMC neurons by blocking melanocortin receptors, resulting in increased appetite and food intake [11]. PACAP was shown to interact with POMC neurons via PAC1 and VPAC2 receptors and via PAC1 receptors with AgRP neurons [11]. Nguyen and colleagues found that PACAP knockout mice had decreased AgRP mRNA expression levels and increased POMC mRNA levels after fasting and refeeding. In both knockout and wild-type mice, expression of NPY and AgRP was upregulated by feeding after a fasting period. However, AgRP expression was higher in wild-type compared to knockout mice. POMC expression was downregulated after fasting and refeeding, with the extent of the downregulation increased in knockout compared to wild-type mice. An ICV injection of PACAP6-38 decreased body weight, food consumption, and AgRP expression after fasting and refeeding in wild-type mice, but no changes in POMC expression [11]. These results suggest that PACAP may act as a regulator of anorexigenic and orexigenic peptides in the hypothalamus. Considering that the authors measured the expression of these peptides, further studies are needed to determine if the level of these peptides and especially the release of these peptides would be altered by endogenous or exogenous PACAP.

PACAP was reported to decrease NPY-stimulated food and water intake in male Sprague-Dawley rats [57]. An earlier study found that PACAP (2.5  $\mu$ g, ICV) decreased food consumption and increased locomotor activity in fasted mice [16]. When mice were administered NPY, food intake increased. Furthermore, a combination of PACAP and NPY induced a decrease in food consumption and increased locomotor activity, suggesting that PACAP and NPY exert opposing effects on food intake in the brain.

Despite PACAP-induced suppression of NPY-mediated food intake in rats, it did not alter the level of NPY in the hypothalamus [57]. However, further studies using lower doses of PACAP are needed to differentiate whether the effect of PACAP is due to an action of the peptide on food intake *per se* or the peptide selectively alters the NPY-stimulated food intake.

Nakata and colleagues (2004) showed that food intake was reduced in PACAP-deficient mice compared to their wild-type controls. They also examined the underlying mechanism of this regulatory action of PACAP and discovered that NPY mRNA was reduced in PACAP-deficient mice. They also showed that PACAP increased intracellular calcium ( $[Ca^{2+}]_i$ ) in NPY-containing neurons of the ARC [21].  $Ca^{2+}$  is a regulator of neuronal activities; it was shown that orexigenic substances of physiological relevance, such as low glucose, ghrelin, and orexin, induce increases in  $[Ca^{2+}]_i$  in NPY-containing neurons [21]. Given that NPY neurons are affected by glucose and other chemicals, these authors also studied if consumption of a high-carbohydrate diet would be changed in these mice. Their results revealed that consumption of a high-carbohydrate but not high-fat diet was reduced in PACAP knockout than wild-type control mice [21]. Together, these findings suggest that endogenous PACAP influences carbohydrate intake via the NPY-releasing neurons in the ARC.

Per Shioda et al. [58], PACAP can increase or decrease the activity of glutamate ionotropic and metabotropic receptors [59] by activating Src protein tyrosine kinases, suggesting that PACAP regulates caloric intake by activating or inhibiting satiety circuits, like in the VMN; and appetite signals, in the nucleus accumbens (NAc); suppressing homeostatic and hedonic feeding drives which can be helpful towards developing a treatment for obesity and overeating./

AP5, an NMDA receptor antagonist injected into the VMN of male Sprague-Dawley rats at doses ranging from 10 pmol to 1nmol, did not seem to alter the feeding behaviors but blocked the effects of PACAP injected into the VMN on food intake when administered before PACAP injections [60]. Appetite and food intake are also decreased by inhibiting ghrelin and stimulating GLP1 and leptin [20]. Per Adams et al. [61], PACAP's role is to maintain energy balance. PACAP-null mice were not obese or hyperphagic compared to wild-type mice regardless of temperature or diet. PACAP-null mice were initially leaner than wild-type mice if maintained on a regular chow diet and at 21°C. Researchers proposed that the decrease in body mass could not be attributed to hypophagia, increased metabolism, or motor activity but rather sensitivity to cold temperatures in these mice. The reduced weight was due to decreased fat mass, leptin, and insulin levels. Researchers found that PACAP-null mice compared with wild-type mice had an improved response to insulin tolerance but normal glucose tolerance. Furthermore, adult PACAP-null mice were sensitive to cold at 4°C, as they lost temperature faster than wild-type mice, suggesting that the thyroid axis is affected because of an observed reduction in thyrotropin-releasing hormone (TRH) mRNA. The study concluded that the reduced body mass of PACAP-null mice could be reversed with a thermoneutral environment or a high-fat diet.

In the chick, PACAP and CRH both inhibit food intake and induce behavioral changes after central injection, suggesting that the anorexigenic effects may be regulated by CRH pathways [62]. In the goldfish, studies on the effect of CRH on feeding behavior after central injection showed that it acts as a hypothalamic anorexigenic peptide [63]. PACAP was shown to regulate the stress response and affective aspect of pain via hypothalamic and extra-hypothalamic structures. For example, PACAP infusions into the central nucleus of the amygdala (CeA) caused pain hypersensitivity and increased anxiety in an elevated plus maze [64]. Similarly, PACAP infusions in the BNST heightened acoustic

startle [25], increased peripheral corticosterone release [65], caused an anorexic phenotype [53], and reinstated cocaine-seeking behavior [66].

The endogenous PACAP/PAC1 receptor system may also be involved in the regulatory action of leptin [39]. Animals injected with leptin into the VMN ate less than controls 3 h after injection, and this effect was blocked by PACAP6–38. Administration of PACAP6–38 alone in the VMN had no effects on food intake or body weight. Leptin administration also increased body temperature 20 h after injection. This response was also reversed by PACAP6–38, given before leptin in the VMN. These results suggest leptin may exert some of its action via the PAC1R activation in the VMN.

Researchers found that PACAP mRNA was co-localized with brain-derived neurotrophic factor (BDNF) mRNA and that PAC1R mRNA was co-expressed with BDNF and the leptin receptor [39]. When PACAP was administered in the VMN, researchers observed STAT3 phosphorylation and increased BDNF and SOCS3 mRNA levels, indicators of leptin receptor activation. These effects of PACAP and leptin were blocked by PACAP6–38, suggesting that PACAP is downstream of leptin and mediating its action on food intake. We propose that there may be a positive feedback loop, where central PACAP release causes leptin release from the periphery and leptin in turn activates the SF-1/PACAP expressing neuron in the VMN, resulting in excitation of POMC neuron [37; 67].

Bath application of PACAP increased neuronal activity, although administration of leptin had a bidirectional effect in which cells that generally fired less often showed an increase in firing rate. In contrast, cells that fired more often showed a decrease in firing rate after administration of leptin. Administration of PACAP6–38 attenuated the effects of leptin on firing and entirely blocked the stimulatory effects of PACAP on neuronal activation.

## **6. The role of PACAP receptor(s) in the regulatory action of PACAP on feeding**

PACAP activates PAC1 receptors with higher affinity than VIP. On the other hand, PACAP and VIP exhibit similar affinity toward VPAC1 and VPAC2 receptors. The PACAP and PAC1 system is thought to play a key role in feeding and energy homeostasis in the hypothalamus [68]. Still, few studies assessed the role of extrahypothalamic PACAP in these processes. PACAP was shown to increase POMC and MC4R mRNA levels in the ARC. The melanocortin system was reported to mediate the central effects of PACAP on thermogenic sympathetic and digestive parasympathetic outflow [69]. Based on this data, it is speculated that PAC1 receptors are found presynaptically on POMC neuronal terminals to increase the synthesis of  $\alpha$ -MSH.

As stated above, ICV PACAP6–38 injection did not affect food intake in mice deprived of food for 18 h but attenuated the inhibitory effect of PACAP on feeding during the 60-min observation period [34]. It was also found that the PACAP antagonist, PACAP6–38, attenuated the inhibitory effects of PACAP on feeding, a response that was not mimicked by VIP, indicating that PACAP has an anorexigenic action through the PAC1 receptor. In a study stated above [21], it was concluded that PACAP and VIP increased depolarization of NPY neurons of the ARC via PAC1 and VPAC2R dose-dependently, resulting in reduced carbohydrate intake by promoting glucose output [49], thus preventing hypoglycemia and lack of energy in PACAP deficient mice. These findings suggest that PACAP has dual insulinotropic and glucagonotropic effects to regulate energy and glucose homeostasis. Given that PACAP could act not only on PAC1 but also VPAC1 and VPAC2 receptors and that PACAP6–38 could activate VPAC2 receptors [70], further studies are needed to characterize the receptor type involved in this process.

## 7. Signaling involved in the modulatory action of PACAP on food intake

According to a study by Dore and colleagues, PACAP released after stress exposure induces CRF expression via the activation of PAC1 receptors in the PVN and increases in intracellular cAMP/protein kinase A signaling [68]. Therefore, CRF can be a target and mediator of PACAP's actions. However, the weight loss and anorectic effects of PACAP seem to be independent of CRF. Researchers found that anorexigenic effects of PACAP were not blocked by the CRF receptor antagonist D-Phe-CRF(12–41) but attenuated by SHU9119, a melanocortin receptor (MCR3/MCR4) antagonist, and by a tyrosine kinase B (TrkB) inhibitor k-252. These observations suggest that the anorexigenic effects of PACAP may be mediated by the melanocortin and TrkB/BNDF pathways [56].

Tyrosine phosphorylation of the NMDA receptor by PACAP is associated with increased Src family kinase activity [71; 72]. The PACAP/PAC1 receptor signaling-induced tyrosine phosphorylation of GluN2B subunit in the NMDA receptor through Src kinase activation plays an important role in food intake regulation. As stated above, PACAP influences glutamate signaling via the N-methyl-D-aspartate receptors (NMDA) via the Src protein tyrosine kinases, suggesting that the Src-NMDA receptor is also a target of PACAP in the VMN to produce hypophagia. This view was based on the finding that VMN PACAP injections produced hypophagia and GluN2B tyrosine phosphorylation [60]. The state of phosphorylation of NMDA by Src kinases is also essential in this regard, as protein phosphatase-1 (PP1), which inhibits the tyrosine phosphorylation of the NMDA receptor, reduced the hypophagic effect of PACAP. PACAP signaling may also regulate  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, as PAC1 receptor-dependent potentiation of AMPA receptors at low PACAP concentrations and VPAC2R depression of AMPA receptors at high concentrations of PACAP in the hippocampus [73]. Also, in the amygdala, the PACAP-PAC1 receptor signaling increases due to the AMPA receptor activity.

## 8. The action of PACAP on homeostatic and hedonic aspects of feeding

Homeostatic food intake is driven by hunger, whereas hedonic food intake is driven by palatability. The lateral hypothalamic area (LHA), containing receptors for various neurohormones and neurotransmitters including GABA, TRH, orexin, and hypocretin, proves to be an area in which the homeostatic and hedonic pathways interact. It was shown that homeostatic food intake is mediated by ARC and PVH neurons, whereas hedonic food intake is mediated by reward systems within the mesolimbic dopaminergic neurons. Researchers found that homeostatically driven food intake in the LHA is attenuated by DA signaling within the basal ganglia. Decreasing D2R and increasing DA release activated reward pathways within the VTA responsible for these hedonic mechanisms. Researchers found that activation of AgRP neurons in the LHA, which is thought to co-release GABA, and inhibitory projections from the BNST to the LHA both prove to further drive hedonic food intake. The NAc may also play a role in hedonic food intake, as it was shown to have efferent and afferent projections to the LHA and ARC, and DA inputs and outputs to the VTA.

A previous study [39] examined the effect of PACAP on the hedonic and homeostatic aspects of feeding. These authors developed a binge-eating paradigm where a large amount of food is consumed within a short time to study the neuroanatomical site of action of PACAP in regulating homeostatic and hedonic drives of food intake. It was found that injection of PACAP into the VMN decreased hunger-mediated food consumption, while injection of the peptide into the NAc did not influence this response.

In contrast, local injection of PACAP into the NAc decreased intake of high-carbohydrate and high-fat foods. This study suggests that PACAP within the VMN reduces the homeostatic aspect of feeding, while injection within the NAc suppresses the hedonic aspect of eating.

Researchers examined whether PACAP excites POMC neurons via PAC1 receptor mediation and TRPC channel activation, a potential mechanism through which PACAP could regulate homeostatic aspects of food intake. In recordings from POMC neurons PACAP induced depolarization and increased firing in the voltage clamp. These postsynaptic actions were abolished by PAC1 receptor antagonists, suggesting that PAC1R is involved in these actions of PACAP. They also showed TRPC channels, phospholipase C, phosphatidylinositol-3-kinase, and protein kinase C are involved in this process. This stimulatory effect on POMC neurons is potentiated by estradiol and attenuated under conditions of diet-induced obesity/insulin resistance [67].

PACAP normally inhibits food intake via a negative feedback loop. However, it has been proposed that, in an addictive state, PACAP acts through a positive feedback mechanism to stimulate food intake and promote drug and food addiction cycles [for a review, see [38]]. The limbic system may play a functional role in the actions of PACAP on food intake since PACAP is stimulated by chronic stress in the BNST and PVN. Chronic stress can lead to anxious and depressive-like moods that trigger the hedonic mechanism of food intake and continue to promote addictive cycles [38]. However, more recent work from Wagner's research team showed that PACAP nerve terminals were optogenetically stimulated in the VMN and Channel Rhodopsin-2 (Chr2) expression was found in the cell bodies of PACAP neurons localized in the VMN and terminals of PACAP neurons in the VTA [74]. Optogenetic stimulation and patch clamp recordings of this PACAP pathway showed that PACAP neurons in the VMN inhibits A10 dopamine neurons within the VTA. Consistent with this, they showed wild-type male mice fed on an intermittent HFD showed increased anticipatory locomotion, caloric intake, heat production, meal size, bout duration and O<sub>2</sub> consumption. PACAP injected into the VTA (30 pmol; 0.2 L) reduced these binge-eating behaviors in males. Although binge-eating caused increased anticipatory locomotion, caloric intake, and O<sub>2</sub> consumption in ovariectomized female mice, PACAP failed to alter these effects in these mice. Intake of regular chow was not altered in mice of either sex.

### Concluding Remarks

A growing body of evidence suggests that PACAP regulates food intake in several species, including fishes, chicks, and rodents. This action of PACAP is primarily via the PACAP-preferring (PAC1) receptor, as the action of PACAP is absent in mice lacking PAC1 receptors or in rodents treated with the PAC1 receptor antagonist PACAP6-38. The anorexic effect of PACAP involves different brain regions with a selective action of the peptide on the homeostatic aspect of food intake into the VMN in the hypothalamus and NAc as the neuroanatomical site mediating the effect of PACAP on the hedonic aspect of food intake. However, further research is needed to define the role of other brain regions, such as VTA and nucleus tractus solitarius, in the regulatory action of PACAP on different aspects of food intake. Additionally, the underlying mechanism of the effect of PACAP in suppressing food intake, particularly its actions on affecting the expression and release of orexigenic and anorexigenic peptides in the hypothalamus, needs to be determined. PACAP appears to be a potential target to develop medications to alter energy and glucose metabolism and possibly binge eating. However, developing medications with selective actions on energy homeostasis without affecting the stress response and cardiovascular and other systems may be a difficult task.



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Table 1: Summary of all the studies included in this review with subjects used, drug dose and route of administration, and their findings

Study By	Subject(s)	Drug Dose Route	Category	Brain Area / Peptide / Peptide precursors, etc.	Findings
Adams BA, Gray SL, Isaac ER, Bianco AC, Vidal- Puig AJ, Sherwood NM (2008)	Male and female C57BL/6 mice	N/A (Null vs WT)	Role of endogenous PACAP	Hypothalamic nuclei	KO had decreased body weight compared to WT from increased insulin sensitivity and increased fat tissue, which was reversed if subjects were given a high-fat diet or in a thermoneutral environment. At 21 C, knockout's body weight could not be explained by decreased food intake, increased metabolic rate, or increased locomotor activity. Post-acute cold challenge, KO showed decreased levels of hypothalamic TRH mRNA levels, brown adipose tissue type 2 deiodinase, and brain deiodinase as compared to WT.
Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW (2002)	Male Sprague Dawley rats	Infusion of CPG, GVIA, APICA, APDC, TBOA	Neuronal function of Glutamate	Glutamate	Blocking glutamate release from a cystine-glutamate antiporter decreases glutamate levels in the rat striatum, but this effect was not replicated by blocking voltage-dependent Na <sup>+</sup> and Ca <sup>2+</sup> . Activity of the cystine-glutamate antiporter is negatively regulated by (mGluR2/3) via a cAMP dependent manner. Extracellular glutamate derived from the antiporter was shown to regulate extracellular levels of glutamate and dopamine. Infusion of the mGluR2/3 antagonist (RS)-1-amino-5-phosphonoindan-1-carboxylic acid (APICA) increased extracellular glutamate levels, and previous blockade of the antiporter prevented the APICA-induced rise in extracellular glutamate and led to increases in extracellular dopamine.
Bernier NJ, Bedard N, Peter RE (2004)	Male and female goldfish	Cortisol Injection, IP Cortisol	Effects on Cortisol on NPY and CRH	CRH, NPY, CRH, telencephalon- preoptic and hypothalamic regions	Daily food intake was elevated in the low cortisol diet group Growth rate was lowest in High cortisol diet group (HCDG), intermediate in the low cortisol diet group (LCDG), and highest in the controls. Feed conversion efficiency was decreased in both experimental groups. After 3 weeks on the diets, the LCDG showed increases in NPY and decreases in CRH mRNA levels in the telencephalon-preoptic brain region. The HCDG was

					characterized by a decrease in CRF mRNA but no difference in NPY levels. IP injection of cortisol increased in NPY and decreased CRF mRNA levels in the telencephalon-preoptic region.
Burgos JR, Iresjö BM, Smedh U (2013)	Male Sprague-Dawley rats	ICV PACAP, ICV CART	PACAP and CART on Food Intake	PACAP6-38, CART	PACAP reduced the ability of CART to decrease food intake and body weight (without altering motor activity) Authors concluded that there may be multiple CARTp receptors.
Chance WT, Thompson H, Thomas I, Fischer JE (1995)	Male Sprague-Dawley rats	IHT Injections of CRG, PACAP, and NPY	Effect of PACAP on NPY-dependent food intake	NPY, PACAP, Hypothalamus	NPY administration increased food intake but PACAP and NPY decreased food intake and increased locomotor activity, Despite PACAP-induced suppression of NPY-mediated food intake in rats, it did not alter the level of NPY in the hypothalamus.
Chang et al (2020)	Male and female mice	IV	Effect of PACAP on POMC Neurons in mediating Homeostatic Food Intake	PACAP, CNO, E2, ETOH, STX, PACAP6-38	PACAP stimulated POMC neurons, but results were attenuated in HFD mice. In vivo experiments revealed that intra-arcuate nucleus Chemogenetic and optogenetic stimulation of VMN and ARC PACAP Neurons decreased energy intake and increased energy expenditure.
Choi DC, Evanson NK, Furay AR, Ulrich-Lai YM, Ostrander MM, Herman JP (2008)	Male Sprague-Dawley rats	Infusion of ibonerate	Anterior BNST affects HPA Axis mediation		Chronic variable stress model reduced body weight, adrenal hypertrophy, thymic involution, and enhanced CRH mRNA in PVN, which wasn't affected by BST lesions. The lesions of the anteroventral BST elevated plasma ACTH and corticosterone responses to novel restraint in the rats previously exposed to CVS. Anterior BST plays very different role in integrating acute stimulation and chronic drive of the HPA axis, perhaps mediated by chronic stress-induced recruitment of distinct BST cell groups or functional reorganization of stress integrative circuits.
Dore R, Iemolo A, Smith KL,	Male Wistar rats	ICV PACAP (PACAP-38) and	PACAP and CRF signaling pathways in PVN	PACAP and CRF signaling pathways in PVN	PACAP released after stress exposure induces CRF expression via the activation of PAC1 receptors in the PVN and increases in intracellular cAMP/protein kinase A signaling. However, the

Wang X, Cottone P, Sabino V (2013)		ICV D-Phe-CRF (12-41)			weight loss and anorectic effects of PACAP 29 seem to be independent of CRF.
Gargiulo AT, Curtis GR, Barson JR (2020)	REVIEW ARTICLE	REVIEW ARTICLE	Food and Drug Addiction Alters PACAP responses	PACAP27, PACAP38, PAC1 R, VIP, VPAC1, VPAC2	Chronic exposure to tasty solutions induces changes in the expression of the splice variants of the PAC1 receptor, and thus changes in PAC1R binding and signaling. In areas of the brain in which both PAC1 and VPAC receptors 11 were present, this change of the variant of PAC1 receptor may favor signaling towards either type of receptor.
Gray SL, Cummings KJ, Jirik FR, Sherwood NM (2001)	Male mice	IV Glucose (ITT)	Role of PACAP in lipid and carbohydrate metabolism	PACAP, Insulin	Glycogen levels were reduced in KO compared to WT. Fasting glucose level was lower with a concomitant increase in insulin levels in KO compared to WT. There was also an increase in cholesterol, triglycerides, and free fatty acids.
Green BD, Irwin N, Cassidy RS, Gault VA, Flatt PR (2006)	Unspecified ob/ob mice	IV PACAP27, IV PACAP 6-38, IP PACAP27, IP PACAP 6-38, IP Glucose, IP Insulin	Role of endogenous PACAP in glucose and insulin levels in ob/ob mice	PACAP27, PACAP38, PACAP 6-38, Insulin	Glucose levels were significantly elevated at 30 and 60 minutes after a glucose challenge in mice injected with PACAP6-27 compared with mice treated with saline. It was also found that once-daily injection of PACAP6-27 for two weeks led to impaired glucose tolerance, the exaggerated glycemic response to feeding with no change in insulin release. Plasma glucagon, triglycerides, total cholesterol, HDL levels were unchanged.
Grinevich V, Fournier A, Pelletier G (1997)	Male rats	ICV PACAP (4 microg/kg b.wt.); IV PACAP (100 microg/kg. b.wt.)	Role of PACAP on CRH Neuron	CRF, PACAP	D-Phe-CRF, a CRF antagonist, does not block PACAP induced anorexia. PACAP raises plasma corticosterone levels, but this effect is not altered by the CRF receptor antagonist (PACAP can cause adrenocorticotrophic hormone (ACTH) secretion directly without causing activation of the hypothalamic pituitary adrenal (HPA) axis).

Hammack SE, Cheung J, Rhodes KM, Schutz KC, Falls WA, Braas KM, May V (2009)	Male Sprague–Dawley rats	PACAP 38 Infusion ( 0, 0.1, 0.5 or 1 µg PACAP38 (0.5 µl/side))	Effect of Stress on VIP, VPAC receptor, PAC1 R, PACAP, TrkB, and BDNF levels	BNST	Chronic stress increased PACAP and PAC1 R BDNF, and TrkB receptor mRNA expression in BNST (VIP and VPAC are unaltered). PACAP38 infusion localized in dBNST induced hypophagic response.
Hannibal J, Georg B, Fahrenkrug J (2016)	Male and female mice	N/A (WT vs Knockouts)	Role of food in anticipatory activity	PACAP	KO and WT entrained to FPP and SPP were placed on a restricted feeding diet, KO mice showed an earlier onset of FAA, greater weight loss, and increased activity than WT at the 10-lux light intensity. Both KO and WT had greater FAA in the SPP 10 and 300 lux, and FPP 300 lux light intensities than FPP 300 intensity.
Hurley, M. M., et al. (2016)	Male Sprague-Dawley rats	PACAP 50 pmol/0.25ul/side/ AMPA 74.5 ng/side / Intra VMN administration	Role of PACAP in obesity	VMN / PACAP, AMPA	PACAP mimicked actions of GABA in the Nac by reducing hedonic feeding but it mimicked AMPA into the VMN by decreasing homeostatic feeding.
Iemolo, A., et al. (2015)	Male Wistar rats	PACAP 0-1ug / rat in the CeA and BIA	Role of PACAP in response to stress	CeA / PACAP	PACAP in the CeA exerts anorectic effects via melanocortin and TrkB.
Jing-Jing Liu, Diptendu Mukherjee, Doron Haritan, Bogna Ignatowska-Jankowska, Ji Liu, Ami	REVIEW ARTICLE	REVIEW ARTICLE	Effects of PACAP on Hedonic Food Intake	ARC, LHA, VTA, NAc, GABA, DA, POMC, NPY, AgRP	Researchers observed the interaction between food intake and reward circuits in the NAc and VTA.

Citri, Zhiping P. Pang (2015)					
Jozsa, R., et al. (2006)	Male Wistar rats and broiler chickens	N/A	Measure levels of PACAP and VIP in response to starvation	hypothalamus, telencephalon, brainstem	PACAP and VIP are involved in food regulation via different mechanisms.
Kiss, P., et al. (2007)	Male and female rats	N/A	Role of Endogenous PACAP in food and water deprivation in various brain areas	PACAP38/ Hypothalamus, Brainstem, and Telencephalon	Found that PACAP levels were changed in a sexually dimorphic manner in the hypothalamus, brainstem, and telencephalon in mice after food and water deprivation.
Kocho-Schellenberg, M., et al. (2014)	Male and female Sprague-Dawley rats	ICV injection of 1.0 µg/0.25 µl PACAP38	PACAP BNST localization on food/water intake	PACAP38, BNST	Infusion of PACAP38 in the posterior (but not anterior) BNST rats induced hypophagic effects.
Kohno, D., et al. (2003)	Male Sprague-Dawley rats	IP Ghrelin, Infusion of Leptin, Superfusion of Orexin	Effect of Ghrelin on NPY in ARC	Leptin, Ghrelin, Orexin, NPY, ARC	Ghrelin interacts with NPY in the ARC, inducing a Ca influx through PKA and N-type Ca (2+) channel-dependent mechanisms. This interaction maybe modulated by neuropeptides and hormones like orexin and leptin to induce effects on feeding behavior.
Lugo, J. M., et al. (2010)	Unspecified African catfish, Nile tilapia	IP PACAP, IP VIP	Role of GH on PACAP and VIP effects on food intake	PACAP, VIP, GH	IP injection of PACAP and VIP increased body weight via GH through protein increase and lipid mobilization. Injection of VIP at a higher dose however did not attenuate food consumption.
Matsuda K, et al. (2006)	REVIEW ARTICLE	REVIEW ARTICLE	Effect of PACAP and VIP in feeding	VMN, ARC, PVN, PACAP, VIP, NPY, AgRP, CRH, CART, leptin, orexin	Looks at role of PACAP and VIP in feeding in vertebrates., as well as the role of orexin, NPY, AgRP, melanin-concentrating hormone, proopiomelanocortin-derived peptides, galanin, galanin-like peptide, ghrelin, CRH, cholecystokinin, CART and leptin.

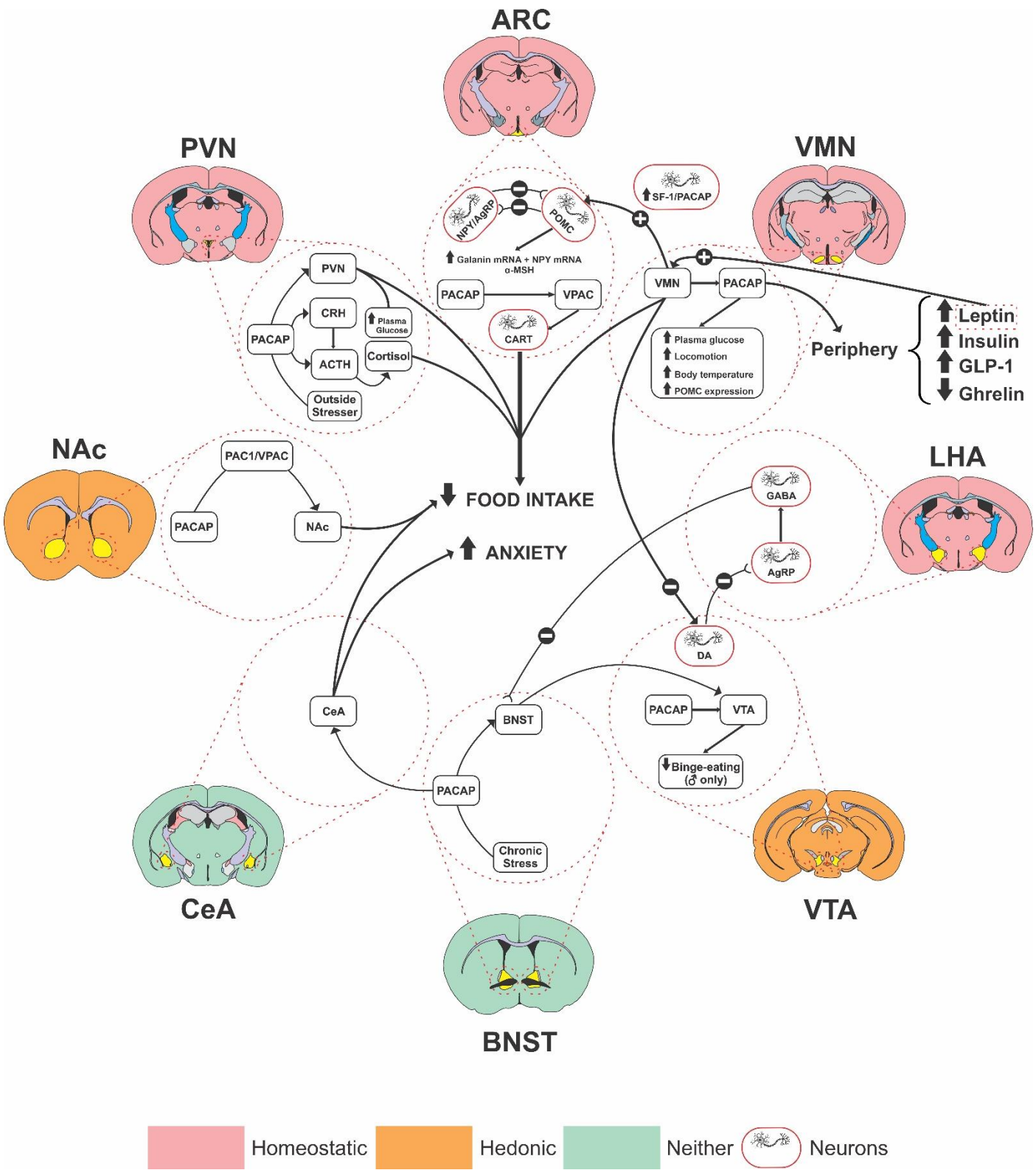
Matsuda, K., et al. (2005)	Unspecified goldfish	IP VIP, ICV VIP, IP PACAP, ICV PACAP	Effect of PACAP and VIP in feeding	PACAP, VIP	ICV and IP PACAP and VIP on food intake in the goldfish, Researchers found that food intake was reduced by ICV PACAP (11 or 22 pmol/g body weight) or VIP (11 or 22 pmol/g) They also found that IP PACAP (44 or 88 pmol/g) or VIP (22 or 44 pmol/g) also decreased food intake.
Mizuno, Y., et al. (1998)	Male Sprague-Dawley rats	ICV	PACAP and involvement of hypothalamic neuropeptide gene expression	Hypothalamus	In PACAP-treated rats, NPY mRNA in the ARC and galanin mRNA levels in the PVN increased, and CRH mRNA levels in the PVN decreased. In rats fasted for 72 h, NPY mRNA levels increased, and CRH mRNA levels decreased. Researchers thus found that PACAP is not mediated by NPY or CRH, and that it increases galanin synthesis.
Morley JE, H. M., Morley PM, Flood JF (1992)	Male TAC:SW mice	ICV	Effect of PACAP on food intake	PACAP, NPY	Purpose of the study: examine ICV injection of PACAP to examine role of cAMP in food intake. When mice were fasted, researchers observed a decrease in food intake in icv injection of 2.5 uG of PACAP but not 1ug. When mice were fasted, researchers observed an increase in locomotor activity of mice when administered 2.5 uG of PACAP compared to vehicles. Mice that were not food deprived were placed in three conditions (PACAP+NPY, saline, or NPY). NPY increased food intake while PACAP and NPY decreased food intake.
Mounien, L., et al. (2006)	Male Swiss albinos CD1 and C57BL/6	Central injection	Expression of PACAP by NPY in ARC	PACAP, NPY, PAC1R, VPACR	Researchers found that a significant amount of NPY neurons express PAC1-R or VPAC2-R mRNA, suggesting that PACAP may regulate the activity of NPY, and the inhibitory effect of PACAP on food intake may be affected by NPY neurotransmission.
Mounien, L., et al. (2009)	Unspecified Rat	ICV	Role of PACAP through Melanocortin system	VIP, PACAP, SHU9119	Researchers found that a high proportion of POMC neurons express PACAP receptors. PACAP may inhibit food intake through a POMC-dependent mechanism. The study concluded that PACAP, may block food intake through a melanocortin-

					dependent pathway and that it is essential for glucose metabolism.
Nakamachi, T., et al. (2019)	Male and female adult short-fin strain zebrafish	ICV	Effect of PACAP on Anorexigenic Action	PACAP1, PACAP2	Researchers observed zebrafish to find that PAC1Rs mRNAs were dominantly expressed in the zebrafish brain. Their results suggested that the expression levels and distribution of duplicated PACAP and PAC1R genes are different in zebrafish, but the effects of PACAP that induce hypophagia, are similar to those seen in other vertebrates.
Nakata, M., et al. (2004)	Male Sprague–Dawley rats	Superfusion	Effect of PACAP on carb intake and NPY in the ARC	PACAP, VIP, NPY, Maxadillian,	Nakata and colleagues showed that NPY mRNA was reduced in PACAP-deficient mice. Given that NPY is involved in carbohydrate intake, these authors also found that carbohydrate intake was reduced in PACAP deficient mice. However, consumption of high-fat diet did not differ between PACAP wild-type and knockout mice. Superfusion of PACAP and VIP causes increases in calcium levels (firing) in a moderately dose dependent manner of NPY Neurons most of the time, as shown by in being immunoreactive in 21 of the 29 NPY neurons, (suggesting that there are potentially other pathways and that the that NPY neuron is not the sole target for PACAP in the ARC).
Nguyen, T. T., et al. (2020).	Male CD1	ICV	Effect on PACAP in VMH on AgRP	PACAP. VIP 6-28, PACAP6-38, AgRP, VMH	Discusses ICV injection of PACAP in mice (can discuss before Zebrafish icv article). ARC contains AgRP/NPY neurons [promote food intake during fasting] and CART/POMC [activated by satiety]. PACAP regulates feeding in VMH by modulating the expression of AgRP. Knockout mice who had fasted for 2 days had significantly lower food consumption during refeeding than in the PACAP (+/+) mice. Researchers also found that the nocturnal and daily food intake of knockout mice was reduced as compared to wild-type mice, but food intake during the day showed an increase in knockout.

Nikki Le (2021)	Male and female C57BL/6 mice	Site specific injection	Effect of PACAP on hedonic feeding	VMN, VTA, DA	Observed the effects of site-specific injection of PACAP into VMN and VTA on hedonic food intake as well as activity levels of DA neurons.
Ohgushi, A., et al. (2001)	Male Broiler chicks	ICV	Feeding and locomotion of CRF in chicks	CRF	Researchers found that CRF acts in the CNS to decrease food intake and increase locomotion in the chick.
Rachel Changa, Jennifer Hernandezb Cassandra Gasteluma, Kaitlyn Guadagnob Lynnea Pereza Edward J. Wagner (2019)	Male and female eGFP-POMC mice, PACAP-Cre mice	Site specific injection	Effect of PACAP on POMC Neurons in mediating Homeostatic Food Intake	POMC, TRPC channels, VMN, ARC	PACAP activates PAC1 R and TRPC5 channels at the VMN and ARC, which is mediated by estradiol, although these effects aren't seen in HFD mice.
Resch, J. M., et al. (2011)	Male Sprague-Dawley rats	Microinjections	Effect of PACAP in VMN on food intake and thermogenesis	PACAP, VMN	The study found that PACAP input to the VMN can influence energy homeostasis.
Resch, J. M., et al. (2013)	Male Sprague-Dawley rats	Microinjections	PACAP regulates feeding	VMN, PVN, PACAP. VIP 6-28, PACAP6-38	When PACAP was injected into both the PVN or VMN, food intake, as measured by the latency in consuming the first meal and the rate of eating, was attenuated in Sprague-Dawley rats as compared to the vehicle. When injected in PVN, rats showed strong decreases in meal size, duration of the meal, and total time spent eating. These effects were reversed by PAC1R antagonists but not VPAC antagonists. Researchers also found that PACAP administration in both the PVN and VMN increased plasma glucose concentration but did not affect

					pancreatic hormone levels. Upon injection of PACAP in the VMN, core body temperature and spontaneous locomotor activity in rats were elevated as compared to controls. These results were reproduced using maxadilan, a PAC1R agonist, but not after VIP injections in either brain regions. (Resch et al 2013).
Resch, J. M., et al. (2014)	Male Sprague-Dawley rats	Injection	NMDA mediates PACAP in VMH	VMN, glutamate, Src kinase, PACAP	These results showed that PACAP in the VMN increases glutamate signaling through NMDA receptors and phosphorylation of the Src kinase to regulate food intake.
Roman, C. W., et al. (2012)	Male Sprague-Dawley rats	Infusion	Effect of BNST on stress and weight Gain	BNST, NMDA	Researchers found that two mechanisms affect the stress induced weight gain, and during chronic stress, the BNST is involved, worsening the symptoms of stress.
Sherwood NM., et al (2007)	REVIEW ARTICLE	REVIEW ARTICLE	Effect of PACAP on reproduction, energy homeostasis, and brain development	PACAP, VPAC, insulin, glucagon	Researchers observed the effect of PACAP knockdown with morpholinos on early brain development in zebrafish. Also reviewed the role of PACAP as a modulator of reproduction in mice and its role in energy homeostasis. It was found that PACAP is crucial for zebrafish brain development and reproduction, but not for body mass or food intake in mice maintained near thermoneutrality.
Tachibana, T., et al. (2003)	Male Chicks	ICV	Effect of various peptides on food intake	VIP, PACAP, GRF, and GLP-1	VIP, PACAP, GRF, and GLP-1 all decreased food intake in chicks at a 12 pmol dosage, but VIP and PACAP with less potency.
Tachibana, T., et al. (2004)	Male Chicks	ICV	Effect of CRH on PACAP and VIP in chicks	PACAP, VIP, astressin, PACAP6-38	Researchers found that ICV injections of PACAP and VIP increased plasma corticosterone concentrations, but blocked through administration of astressin, suggesting that CRF neurons modulate PACAP and VIP induced hypophagia.
Vu, J. P., et al. (2015)	Unspecified C57BL/6	IP	Effect of PACAP on other neurotransmitters	PACAP, Ghrelin, GLP-1, Leptin, Insulin, Glucagon, PYY	Researchers found that WT mice that were administered PACAP showed decreased food intake and ghrelin. KO did not show any change in food intake upon PACAP administration. In fasted KO, ghrelin, GLP-1, insulin, and leptin and

			to decrease food intake		postprandial levels of active ghrelin and insulin were different than that of KO.
Yada, T., et al. (1994)	Unspecified Rat	Not Specified	Effect of PACAP on Insulin Secretion	PACAP, VIP, Insulin	PACAP at low doses increased insulin release from rat islets in a glucose-dependent manner. And increased calcium concentration in pancreatic beta-cells. VIP also increased calcium concentration but was less potent.
Yada, T., et al. (2000)	Female GK rats and C57B/6J mice	IP	PACAP effect in blood glucose in GK rats (diabetic) and normal high fat diet mice (glucose intolerant)	PACAP, Insulin	Researchers observed the effects of PACAP of the hyperglycemic rats and in high fat mice. The hyperglycemic rats housed with normal diet showed increased blood glucose levels until three weeks old but significant hyperglycemia at eight weeks, but upon early treatment of PACAP, blood glucose decreased. In high fat mice, IP PACAP decreased glucose levels with no effect on insulin levels.
Yokota, C., et al. (1995)	Male Wistar-lmamichi rat	Infusion	Effect of PACAP on glucose	PACAP, Theophylline, VIP, Glucagon	Researchers found that administration of PACAP increased glucose output, and to a lesser extent, cAMP. Theophylline did not affect these results. On the other hand, in the perfusion experiment with Ca2+ free perfusate, the degrees of increase in glucose output induced by 15 and 40 nM PACAP-27 were significantly reduced. In conclusion, PACAP stimulates glucose output from the perfused rat liver, and Ca2+ rather than cAMP plays an important role in this action as a second messenger
Yu, R., et al. (2008)	Male NIH Mice	IP	Effect of chronic maxadillan on glucose levels and insulin sensitivity	M65, maxadillian, insulin	Researchers found that maxadilan-induced hyperglycemia was blocked by M65. Acute maxadilan decreased feeding and enhanced water intake significantly for the first several days. Chronic maxadilan increased body weight and decreased body fat. Researchers also found that it down-regulated basal plasma glucose and upregulated basal plasma insulin, while increasing glucose tolerance and insulin sensitivity.



**Figure 1:** Schematic Diagram of different brain regions involved in the regulatory action of PACAP on feeding. Arcuate Nucleus (ARC); Bed Nucleus of Stria Terminalis (BNST); Central Nucleus of Amygdala (CeA); Lateral Hypothalamus (LHA); Nucleus Accumbens (NAc); Paraventricular Nucleus of the Hypothalamus (PVN); Ventromedial Nucleus of the Hypothalamus (VMN); Ventral Tegmental Area (VTA)

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