P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.127

Differential Expression of Hypothalamic Anorexigenic and Orexigenic Neuropeptides in Adult Mice

Thanh Trung Nguyen [1], Thi Ngan Mai [2], Phuong Nhung Nguyen [3] and Ha Thi Thanh Nguyen [1,4,*]

[1]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 131000, Vietnam
[2]Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 131000, Vietnam
[3]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 131000, Vietnam
[4]Laboratory of Pharmacology and Drug Development, Center of Excellent Research and Innovation, Vietnam National University of Agriculture, Gia Lam District, Hanoi 131000, Vietnam
***Corresponding author:** nguyenhavet@vnua.edu.vn

Article History: 25-145 Received: 23-May-25 Revised: 09-Sep-25 Accepted: 25-Sep-25 Online First: 21-Oct-25

ABSTRACT

Appetite regulation is a complex physiological process that is controlled by a network of hypothalamic neuropeptides. Among these neuropetides, pituitary adenylate cyclase-activating polypeptide (PACAP) has emerged as a key stimulator, but its role during fasting remains poorly defined. In this study, we assessed the expression of important appetite–related neuropeptides in response to fasting. PACAP has been known as an important factor that regulates feeding behavior through central neuropeptide signaling pathways. Expression levels of cholecystokinin (CCK), melanin concentrating hormone (MCH), orexin, and oxytocin in the hypothalamus of wild-type mice were notably upregulated in response to fasting; however, no significant changes were observed in PACAP−/− mice, indicating that the response is PACAP-dependent. In contrast, amylin expression was downregulated by fasting in both genotypes, suggesting a PACAP-independent, potentially anorexigenic role. Notably, bombesin and neurotensin expression levels remained unchanged across all conditions. Our results demonstrated that PACAP modulates the neuropeptidergic signaling network in a specific and selective manner, and therefore participates in the central regulation of appetite. These findings highlight the peculiar contribution of PACAP in balancing anorexigenic and orexigenic pathways during energy deprivation. Such selective regulation suggests that PACAP acts as a metabolic sensor, integrating fasting signals with hypothalamic centres that control feed intake.

Key words: Pituitary adenylate cyclase-activating polypeptide, Hypothalamus, Appetite, Neuropeptides, Food intake.

INTRODUCTION

The energy homeostasis and behavior of eating are mediated by a complex neuroendocrine network, in which signals from peripheral metabolism are integrated with pathways of the central nervous system (Chen et al. 2025). The hypothalamus is central in this network, integrating signals like leptin, insulin, and ghrelin, coordinating proper reactions by the neuropeotides that either stimulate appetite or suppress hunger, and therefore maintaining the homeostasis of energy (Bombassaro et al. 2024; Basu and Flak 2025).

Fasting can trigger adaptive physiological responses in various animal species, such as geese (Liu et al. 2025), laying hens (Weng et al. 2025), birds (Liu et al. 2024) and dogs (McKenzie et al. 2024). In geese, these diets affect nutrient regulation and energy metabolism (Liu et al. 2025). In laying hens, they alter gut microbiota and liver functions (Weng et al. 2025). In dogs, long-term consumption of high-fat diets leads to metabolic disorders that are similar to age-related changes, including resistance to insulin and dyslipidemia (McKenzie et al. 2024). The findings across a number of species, including both livestock and companion animals, indicate the significance of research about fasting in veterinary medicine. The hypothalamus is vital for the behavior of feeding and the maintenance of energy homeostasis. In addition, hormones such as alpha melanin-stimulating hormone (α-MSH) and

Cite This Article as: Nguyen TT, Mai TN, Nguyen PN and Nguyen HTT, 2026. Differential expression of hypothalamic anorexigenic and orexigenic neuropeptides in adult mice. International Journal of Veterinary Science 15(1): 164-170. https://doi.org/10.47278/journal.ijvs/2025.127

neuropeptide Y (NPY) have been demonstrated to affect the appetite and storage of fat via hypothalamic pathways in several species, including chicks and quails (Liu et al. 2024).

In the literature, the arcuate nucleus (ARC) plays a central role, containing neurons that express orexigenic neuropeptides like NPY and agouti-related peptide (AgRP), as well as anorexigenic peptides such as proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) in the hypothalamus (Joly-Amado et al. 2014; Tran et al. 2022; Song and Choi 2023). These neurons project to other regulatory centers, including the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA), influencing downstream neuropeptides such as corticotropin-releasing hormone (CRH), melanin-concentrating hormone (MCH), orexin, and oxytocin (Schwartz et al. 2000; Ferguson et al. 2008; Iremonger and Power 2025). In addition, peripheral peptides also contribute significantly to appetite regulation. In more detail, cholecystokinin (CCK), amylin (IAPP), and bombesin-like peptides mediate short-term satiety through gut-brain signaling (Ding et al. 2022), while centrally produced neuropeptides such as orexin, MCH, neurotensin, and oxytocin influence both feeding and energy expenditure (Raybould 2007; Miller 2019; Izawa et al. 2025). These neuropeptides function within interconnected networks rather than in isolation. Functional redundancy and compensatory mechanisms often obscure the roles of individual peptides in gene knockout studies (Peng 2019; Nguyen et al. 2020; Rouf et al. 2023).

Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide classified within the VIP/secretin/glucagon family, was initially identified in the hypothalamus because of its capacity to stimulate cAMP signaling in pituitary cells (Fuentes-Fayos et al. 2022). Within its widespread expression in brain regions involved in energy regulation, PACAP is considered a strong candidate for modulating appetite by influencing both orexigenic and anorexigenic pathways (Nguyen et al. 2020; Sureshkumar et al. 2021; Kambe et al. 2023; Vu et al. 2023). No prior study has comprehensively analyzed the effect of PACAP deficiency on the fasting-induced regulation of CCK, MCH, orexin, oxytocin, amylin, bombesin, and neurotensin in adult mice.

To address these issues, the present study investigates the role of PACAP in regulating hypothalamic neuropeptide expression under fasting conditions. PACAP knockout mice were employed in the experiments to elucidate neuropeptidergic circuits that are regulated by PACAP, clarify possible compensatory mechanisms, and further explain how the integration of central and peripheral signals controls appetite. The knowledge would be useful in the development of treatment therapies for metabolic diseases, such as obesity and cachexia.

MATERIALS AND METHODS

Animals

PACAP (−/−) mice (Hashimoto et al. 2001) were used in our experiments. Male CD-1 mice (Japan SLC Inc., Shizuoka, Japan) with an age range from 8 to 13 weeks were separately housed for a minimum of 1 week and during the whole experimental time, with the condition of a 12-hour light/dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.). All procedures involving animals were reviewed and approved by the Experimental Animal Research Committee of Kagoshima University and the Vietnam National University of Agriculture.

PACAP genotyping

The method for genotyping was adapted from a previously published protocol (Hashimoto et al. 2001). Ear punch samples (0.25cm) were obtained from mice and incubated in 50mM NaOH at 95°C for one hour. After incubation, 1M Tris-HCl (pH 8.0) was added to neutralize the samples. PCR amplification was conducted on a thermal cycler (PC-320, ASTEC, Fukuoka, Japan), beginning with a 3-minute denaturation at 95°C, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 59°C, and 2 minutes at 72°C, with a final hold at 4°C. The amplified products were then separated and visualized using 1% agarose gel electrophoresis. The primer sequences employed for genotyping are presented in Table 1.

Fasting–refeeding paradigm and gene expression analysis (RT-qPCR)

In the fasting experiment, mice underwent a two-day period without food. Total RNA was isolated with the Sepasol-RNA 1 Super G kit (Nacalai Tesque, Kyoto, Japan) and cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). qPCR was performed with the Thunderbird SYBR qPCR kit (Toyobo Life Science, Osaka, Japan) on a Thermal Cycler Dice Real-Time System TP800 (Takara Bio Inc., Shiga, Japan). Specific primers targeting amylin, bombesin, CCK, MCH, neurotensin, orexin, and oxytocin were designed using Primer 3 (Koressaar and Remm 2007), along with primers for the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH), as detailed in Table 2. All procedures followed the manufacturer's instructions. Gene expression levels were normalized to GAPDH and presented as relative expression percentages.

Statistical analysis

Data are presented as mean ± S.E.M. Statistical analysis was performed using Student's t-test or one-way ANOVA with Dunnett's or Bonferroni's post hoc tests, as appropriate, using Prism version 4.3 (GraphPad Software, San Diego, CA). Statistical significance was indicated when a p-value was less than 0.05.

RESULTS

Fasting-induced increments in the expression of CCK, MCH, orexin and oxytocin are abolished in PACAP (−/−) mice

In normal mice, mRNA expression levels of CCK, MCH, orexin, and oxytocin in hypothalamic regions, including the paraventricular thalamus (PVT), bed nucleus of the stria terminalis (BNST), and medial preoptic area

Table 1: List of primer sequences used for genotyping

Targets	Expected size (bp)	Forward (5' - 3')	Reverse (5' - 3')
PACAP (+/+)	293	ACCGAAAACAAATGGCTGTC	GGTCCACAAAGTATATCTGTGCATTCTC
PACAP -/-)	1078	ATCTCCTGTCATCTCACCTTGCTCCT	GGTCCACAAAGTATATCTGTGCATTCTC

Table 2: Sequences of primers utilized in RT-qPCR

Genes	Primer names	Sequences (5'-3')	References
Amylin	F	ACC ACT GAA AGG GAT CTT GAG	This study
	R	TTT GTC CAT CTG AGG GTT GCT A	
Bombesin	F	GGT CCT GGC TAA GAT GTA TCC G	
	R	GTC TCT GTC AGC CGC ATA CAG	
CCK	F	CCG CCT GCC CTC AAC TTA	
	R	CAT CAC CAC GCA CAG ACA TAC G	
MCH	F	GCG GTT TCA TGA ACG ATG ATG	
	R	GAT CCT TTC AGA GCG AGG TAA G	
Neurotensin	F	TGG TGT GCC TGA CTC TC	
	R	GCT GAT CTT GGA TGT ATG CAT G	
Orexin	F	CTT TCC TTC TAC AAA GGT TCC C	
	R	TAG AGA CGG CAG GAA CAC G	
Oxytocin	F	GAC CTC GGC CTG CTA CAT CCA G	
	R	GCC GCA GGG GAG ACA CTT G	
GAPDH	F	GAAGGTCGGTGTGAACGGAT	(Kambe et al. 2023)
	R	CTCGCTCCTGGAAGATGGTG	

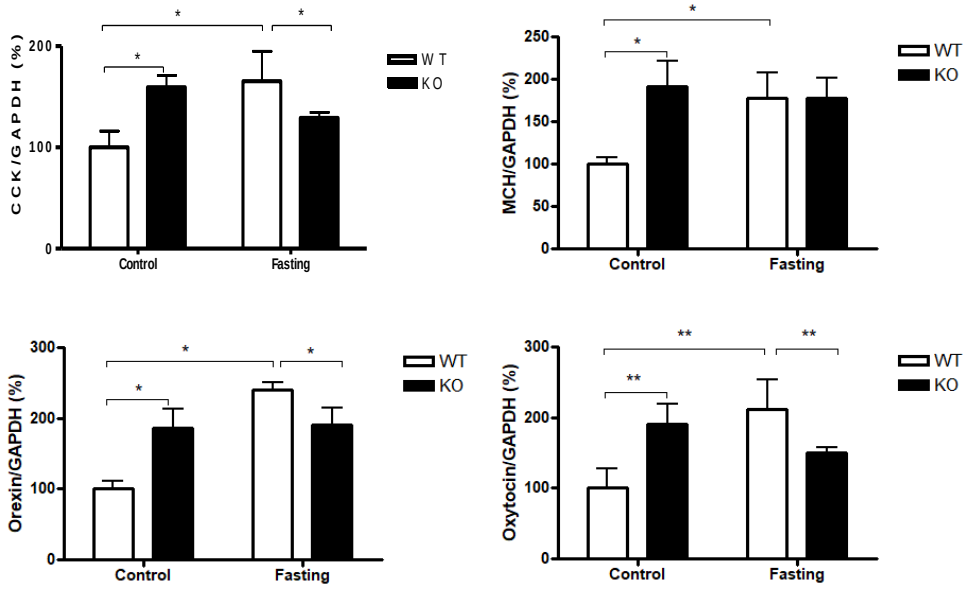

Fig. 1: Fasting-induced increments in the expression of CCK, MCH, orexin, and oxytocin are abolished in PACAP (−/−) mice. RT-qPCR was used to quantify hypothalamic expression of CCK, MCH, orexin, and oxytocin in PACAP (+/+) (white bars) and PACAP (−/−) (black bars) mice under naïve or 2-day fasting conditions (n=8 per group). Data were analyzed by ANOVA with Tukey's post hoc test (*P<0.05, **P<0.01).

(MPOA), were remarkably upregulated as a result of fasting. However, the increment was absent in PACAP (−/−) mice, suggesting the participation of these in the orexigenic response mediated by PACAP. The findings, as shown in Fig. 1, indicate that PACAP neurons in the PVT, BNST, and MPOA may have extensive projections and play crucial roles in regulating feeding behavior.

Fasting-induced decrements in the expression of amylin are reversed in PACAP (−/−) mice

In opposition to the upregulation that fasting induces on the mRNA expression of CCK, MCH, orexin and oxytocin, the level of amylin mRNA in the hypothalamic area was significantly downregulated with fasting. Interestingly, this downregulation was reversed in PACAP knockout (PACAP−/−) mice, indicating that a PACAP-independent mechanism is involved in the suppression of amylin, as shown in Fig. 2. This observation suggests an antagonistic role of amylin in PACAP-mediated orexigenic signaling, and therefore emphasizes its noticeable pathway in the regulation of feeding behavior.

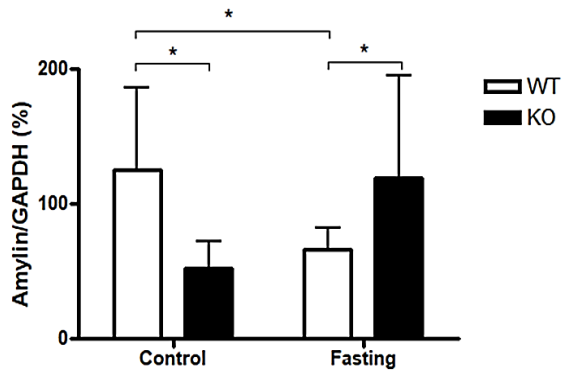

Fig. 2: Fasting-induced decrements in the expression of amylin are reversed in PACAP (−/−) mice. Amylin mRNA levels were measured by RT-qPCR in hypothalamic tissue from PACAP (+/+) (white bars) and PACAP (−/−) (black bars) mice under naïve or two-day fasting conditions (n=8 per group). Data were analyzed using ANOVA followed by Tukey's post hoc test (*P<0.05).

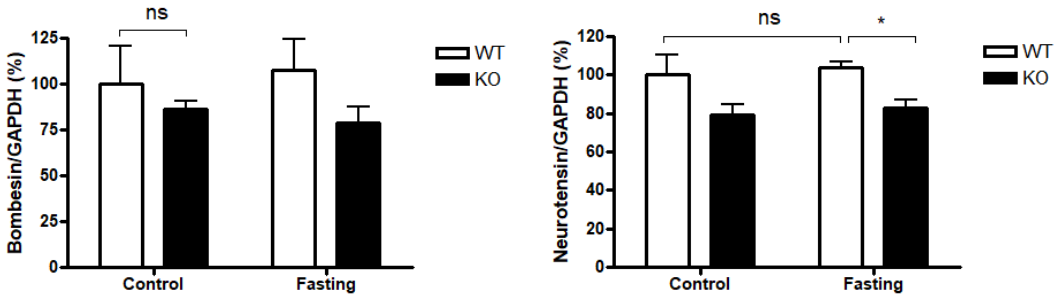

Fig. 3: Fasting-induced lack of change in bombesin and neurotensin expression is preserved in PACAP (−/−) mice. Bombesin and neurotensin mRNA levels were measured by RT-qPCR in hypothalamic tissue from PACAP (+/+) (white bars) and PACAP (−/−) (black bars) mice under naïve or 2-day fasting conditions (n = 8 per group). Data were analyzed by ANOVA with Tukey's post hoc test (*P<0.05, significant; ns, not significant P> 0.05).

Bombesin and neurotensin expression remained unaltered by fasting or PACAP deficiency

In contrast to results on expressions of other neuropeptides, the levels of bombesin and neurotensin in the PVT, BNST, and MPOA remained unaffected by fasting, and there were no significant differences between wild-type and PACAP (−/−) mice. These observations suggest that bombesin and neurotensin are not involved in the changes of feeding behavior induced by fasting, as shown in Fig. 3.

DISCUSSION

Our study has clarified the roles of PACAPs in the modulation of feeding-related neuropeptides during fasting in mice, and specifically focused on important hypothalamic regions that are known to be involved in appetite control. The results demonstrate the essential role of PACAP in the upregulation of several orexigenic neuropeptides in fasting, such as CCK, MCH, orexin, and oxytocin. However, the significant elevation in mRNA levels of these neuropeptides in wild-type mice was abolished in PACAP-deficient (PACAP−/−) mice. The results indicate that PACAP signaling is essential for the transcriptional activation of the genes when responding to the deficiency of energy, and therefore contributes to the orexigenic stimulation that increases food intake after fasting.

In the first place, our results affirm the essential roles of PACAP in the coordination of fasting-induced upregulation in several key orexigenic neuropeptides, including CCK, MCH, orexin, and oxytocin. In wild-type mice, fasting significantly elevated the mRNA levels of these peptides, a response entirely absent in PACAP−/− mice, highlighting the necessity of PACAP signaling in their activation during energy deficiency. Cholecystokinin (CCK), a well-studied gut-brain peptide, plays a crucial role in promoting satiety by reducing meal size through its peripheral actions, such as stimulating gallbladder contraction and delaying gastric emptying (Gibbs et al. 1973; Moran and McHugh 1982; Asim et al. 2024; Rehfeld, 2025). Interestingly, under fasting conditions, its central expression might shift toward stimulating appetite under PACAP regulation.

Melanin-concentrating hormone (MCH), which is mainly expressed in the lateral hypothalamus (LHA), is another potent orexigenic peptide whose expression increases during fasting. MCH promotes feeding and suppresses energy expenditure, likely through its interaction with the HPA axis (Qu et al. 1996; Saito et al. 1999; Al-Massadi et al. 2021; Concetti et al. 2023; Payant et al. 2025). Similarly, orexin A, produced in the LHA, stimulates both feeding and arousal, in addition to regulating thermogenesis (Tsujino and Sakurai 2013; Liu et al. 2020; Maruyama and Ueta 2023). Its role in enhancing appetite is well established (Sakurai et al. 1998), and its elevated expression during fasting reinforces its involvement in maintaining energy balance.

Conversely, oxytocin, a hormone known for its roles in parturition and lactation, also acts within the brain to suppress food intake (Olson et al. 1991; Arrowsmith and Wray 2014; Maier and Brecht 2022; Yukinaga and Miyamichi 2025). Regardless of the anorexigenic nature, fasting induced an increment in oxytocin expression in wild-type mice, but not in PACAP−/− mice, suggesting a more complicated and also contextual role in the modulation of feeding behavior. It might be explained by the fact that upstream in the functions of PACAP is aimed to modulate the hypothalamic oxytocin neurons, as the reaction to metabolic stress, and possibly via the direct signals on PAC1 receptor, or intermediary neuropeptides, like corticotropin-releasing hormone (CRH) or leptin. On the other hand, the integration of metabolic signals within the paraventricular nucleus (PVN) might be impaired by the absence of PACAP, leading to the reduction of oxytocinergic activation in response to fasting. There was also a previous study reporting the crucial contributions of interactions between ventromedial PACAP and dorsomedial galanin in the regulation of appetite by the hypothalamus of mice (Kambe et al. 2023). In general, the results indicate that PACAP plays an important role in the incorporation of various neuroendocrine signals in the response to fasting, through the integration of various appetite-stimulating pathways. This network leads to the increment in feeding motivation and the restoration of energy under fasting circumstances. However, future researches are still needed to clarify the mechanisms responsible for this mode of action.

Notably, amylin shows a distinct expression pattern compared to the orexigenic neuropeptides examined in this study. Its mRNA levels were significantly downregulated during fasting in both wild-type and PACAP−/− mice, suggesting that its decrease is independent of PACAP signaling. This finding aligns with amylin's well-recognized function as an anorexigenic peptide (Roth et al. 2012; Volčanšek et al. 2025; Walker et al. 2025).

Furthermore, amylin is also referred to as amyloid polypeptide (IAPP) that is released with insulin by pancreatic beta cells in response to nutrient intake (Butler et al. 1990; Eržen et al. 2024). It acts as a short-term regulator of satiety by slowing gastric emptying, decreasing meal size, and helping maintain blood glucose levels (Hankir and Le Foll 2025). Research has demonstrated that peripheral amylin administration reduces food consumption, partly by limiting meal size and that prolong treatment can lead to weight loss (Arnelo et al. 1996; Lutz et al. 2000). The reduction in expression of amylin in fasting observed in our study suggests a ameliorative mechanisms that diminish anorexigenic signaling when amounts of energy are decreased, and therefore increasing the feeding desires and supporting the energy restoration. This mode of action contributes to the reduction of satiety signals in cases where the accessibility to nutrients is insufficient. Remarkably, the decrease in levels of amylin was also observed in PACAP-deficient mice, supporting the idea that noticeable upstream regulators participate in the neuropeptide systems controlling appetite. While PACAP is essential for the upregulation of appetite-stimulating peptides, such as CCK, MCH, orexin, and oxytocin in fasting, it is likely that amylin expression is mediated by a different signaling network, which might include the peripheral metabolic signals or brain circuits that are independent from PACAP. These findings indicate the diversity and complication of pathways responsible for food intake in neuroendocrine systems. In addition, observed results signify that the dualism of orexigenic and anorexigenic signals in the modulation of appetite is mediated by separate molecular routes. However, future research is necessary to elucidate the overlap or independence of amylin- or PACAP-involving pathways, in both hunger and satiety conditions. These scientific knowledges might serve as a background to develop the specified targeted therapies applied in related diseases, such as obesity or diabetes.

In contradiction to the results on appetite-regulating peptides, where the expression was suppressed or increased by PACAP genotype and fasting, levels of bombesin and neurotensin in the hypothalamic region remained unchanged, indicating that these neuropeptides are not dependent on the acute regulation generated by energy status or PACAP signaling. Because previous studies have demonstrated that the neurotensins synthesized in hypothalamic nuclei, such as the ARC, PVN, and DMH, have functions in the decrement of food intake and are positively controlled by leptin (Luttinger et al. 1982; Sahu et al. 2001; Primeaux et al. 2021; Fu et al. 2025), we suggest that they might be more involved in the long-term maintenance of energy balance. The stable expression of neurotensin indicates that its roles in feeding might be region-specific or mediated via mechanisms that are different from immediate transcriptional changes. Similarly, unchanged levels of bombesin imply that it is not involved in PACAP-mediated responses to short-term fasting. In contradiction to the results on appetite-regulating peptides, where the expression was suppressed or increased by PACAP genotype and fasting, levels of bombesin and neurotensin in the hypothalamic region remained unchanged, indicating that these neuropeptides are not dependent on the acute regulation generated by energy status or PACAP signaling. Because previous studies have demonstrated that the neurotensins synthesized in hypothalamic nuclei, such as the ARC, PVN, and DMH, have functions in the decrement of food intake and are positively controlled by leptin (Luttinger et al. 1982; Sahu et al. 2001; Primeaux et al. 2021), we suggest that they might be more involved in the long-term maintenance of energy balance. The stable expression of neurotensin indicates that its roles in feeding might be region-specific or mediated via mechanisms that are different from immediate transcriptional changes. Similarly, unchanged levels of bombesin imply that it is not involved in PACAP-mediated responses to short-term fasting.

Conclusion

Taken as a whole, our results clarify the roles of PACAP in the mediation of fasting-induced responses, and also identify the orexigenic neuropeptides that are involved in the process, while, on the other hand, ruling out the dependence of others, such as amylin, bombesin, and neurotensin, on PACAP-regulating signalings. These findings further give an insight into mechanisms of brain neuropeptide signaling in feeding behaviors to adapt to changes in energetic levels, which could be useful to develop targeted therapies applied in the treatment of several metabolic disorders. However, further *in vivo* experiments with animal disease models are still required to assess mechanisms in diseased individuals.

DECLARATIONS

Funding: This research was funded by the Vietnam National University of Agriculture under grant number T2024-09-33.

Acknowledgement: We would like to thank the anonymous reviewers for their thoughtful comments and efforts towards improving our manuscript.

Conflict of Interest: The authors have no conflicts of interest to disclose.

Data Availability: The data will be made available on reasonable request.

Ethics Statement: All procedures involving animals were reviewed and approved by the Experimental Animal Research Committee of Kagoshima University and the Vietnam National University of Agriculture.

Author's Contribution: Thanh Trung Nguyen: Writing – original draft, Investigation, Validation, Funding acquisition, Formal analysis, Data curation, Conceptualization; Thi Ngan Mai: Investigation, Validation, Formal analysis, Data curation; Phuong Nhung Nguyen**:** Investigation, Validation, Formal analysis, Data curation; Ha Thi Thanh Nguyen: Conceptualization, Supervision, Writing – review & editing.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

REFERENCES

Al-Massadi O, Dieguez C, Schneeberger M, López M, Schwaninger M, Prevot V and Nogueiras R, 2021. Multifaceted actions of melanin-concentrating hormone on mammalian energy homeostasis. Nature Reviews Endocrinology 17(12): 745-755. https://doi.org/10.1038/s41574-021-00559-1

Arnelo U, Permert J, Adrian TE, Larsson J, Westermark P and Reidelberger RD, 1996. Chronic infusion of islet amyloid polypeptide causes anorexia in rats. The American Journal of Physiology 271(6 Pt 2): R1654-1659. https://doi.org/10.1152/ajpregu.1996.271.6.R1654

Arrowsmith S and Wray S, 2014. Oxytocin: its mechanism of action and receptor signalling in the myometrium. Journal of Neuroendocrinology 26(6): 356-369. https://doi.org/10.1111/jne.12154

Asim M, Wang H, Waris A, Qianqian G and Chen X, 2024. Cholecystokinin neurotransmission in the central nervous system: Insights into its role in health and disease. BioFactors 50(6): 1060-1075. https://doi.org/10.1002/biof.2081

Basu R and Flak J N, 2025. Hypothalamic neural circuits regulating energy expenditure. In: Litwack, G. (Ed.), Vitamins and Hormones. Academic Press, pp. 79-124. https://doi.org/10.1016/bs.vh.2024.07.004

Bombassaro B, Araujo EP and Velloso LA, 2024. The hypothalamus as the central regulator of energy balance and its impact on current and future obesity treatments. Archives of Endocrinology and Metabolism 68: e240082. https://doi.org/10.20945/2359-4292-2024-0082

Butler PC, Chou J, Carter WB, Wang YN, Bu BH, Chang D, Chang JK and Rizza RA, 1990. Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. Diabetes 39(6): 752-756. https://doi.org/10.2337/diab.39.6.752

Chen J, Cai M and Zhan C, 2025. Neuronal regulation of feeding and energy metabolism: A focus on the hypothalamus and brainstem. Neuroscience Bulletin 41(4): 665-675. https://doi.org/10.1007/s12264-024-01335-7

Concetti C, Peleg-Raibstein D and Burdakov D, 2023. Hypothalamic MCH neurons: From feeding to cognitive control. Function 5(1). https://doi.org/10.1093/function/zqad059

Ding Y, Zhang H, Liao YY, Chen LN, Ji SY, Qin J, Mao C, Shen DD, Lin L, Wang H, Zhang Y and Li XM, 2022. Structural insights into human brain–gut peptide cholecystokinin receptors. Cell Discovery 8(1): 55. https://doi.org/10.1038/s41421-022-00420-3

Eržen S, Tonin G, Jurišić Eržen D and Klen J, 2024. Amylin, another important neuroendocrine hormone for the treatment of diabesity. International Journal of Molecular Sciences 25(3): 1517. https://doi.org/10.3390/ijms25031517

Ferguson AV, Latchford KJ and Samson WK, 2008. The paraventricular nucleus of the hypothalamus - a potential target for integrative treatment of autonomic dysfunction. Expert Opinion on Therapeutic Targets 12(6): 717-727. https://doi.org/10.1517/14728222.12.6.717

Fu W, Lai Y, Li K, Yang Y, Guo X, Gong Q, Zhou X, Zhou L, Liu C, Zhang Z, So J, Zhang Y, Huang L, Lu G, Yi C, Wang Q, Fan C, Liu C, Wang J, Yu H, Zhao Y, Huang T, Roh H C, Liu T, Tang H, Qi J, Xu M, Zheng Y, Huang H and Li J, 2025. Neurotensin-neurotensin receptor 2 signaling in adipocytes suppresses food intake through regulating ceramide metabolism. Cell Research 35(2): 117-131. https://doi.org/10.1038/s41422-024-01038-8

Fuentes-Fayos AC, Alors-Perez E, Jiménez-Vacas JM, Herrero-Aguayo V, Sáez-Martínez P, Lopez-Cánovas JL and Luque RM, 2022. Physiology of the pituitary hormone secretion. In: Pituitary Adenomas: The European Neuroendocrine Association's Young Researcher Committee Overview, Cham: Springer International Publishing, pp. 21-56.

Gibbs J, Young R C and Smith G P, 1973. Cholecystokinin decreases food intake in rats. Journal of Comparative and Physiological Psychology 84(3): 488-495. https://doi.org/10.1037/h0034870

Hankir MK and Le Foll C, 2025. Central nervous system pathways targeted by amylin in the regulation of food intake. Biochimie 229(95-104. https://doi.org/10.1016/j.biochi.2024.10.012

Hashimoto H, Shintani N, Tanaka K, Mori W, Hirose M, Matsuda T, Sakaue M, Miyazaki JI, Niwa H, Tashiro F, Yamamoto K, Koga K, Tomimoto S, Kunugi A, Suetake S and Baba A, 2001. Altered psychomotor behaviors in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP). Proceedings of The National Academy of Sciences 98(23): 13355-13360. https://doi.org/10.1073/pnas.231094498

Iremonger KJ and Power EM, 2025. The paraventricular nucleus of the hypothalamus: A key node in the control of behavioural states. The Journal of Physiology 603(8): 2231-2243. https://doi.org/10.1113/jp288366

Izawa S, Fusca D, Jiang H, Heilinger C, Hausen AC, Wunderlich FT, Steuernagel L, Kloppenburg P and Brüning J C, 2025. Orexin/hypocretin receptor 2 signaling in MCH neurons regulates REM sleep and insulin sensitivity. Cell Reports 44(2). https://doi.org/10.1016/j.celrep.2025.115277

Joly-Amado A, Cansell C, Denis RGP, Delbes AS, Castel J, Martinez S and Luquet S, 2014. The hypothalamic arcuate nucleus and the control of peripheral substrates. Best Practice & Research Clinical Endocrinology & Metabolism 28(5): 725-737. https://doi.org/10.1016/j.beem.2014.03.003

Kambe Y, Nguyen TT, Yasaka T, Nguyen TT, Sameshima Y, Hashiguchi K, Shintani N, Hashimoto H, Kurihara T and Miyata A, 2023. The pivotal role of neuropeptide crosstalk from ventromedial-PACAP to dorsomedial-galanin in the appetite regulation in the mouse hypothalamus. Molecular Neurobiology 60(1): 171-182. https://doi.org/10.1007/s12035-022-03084-y

Koressaar T and Remm M, 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics (Oxford, England) 23(10): 1289-1291. https://doi.org/10.1093/bioinformatics/btm091

Liu X, Gao S, Zhang N, Jin T, Sun X, Luan X, Xu L and Guo F, 2020. The orexinergic neural pathway from the lateral hypothalamus to the nucleus accumbens and its regulation of palatable food intake. Neuropeptides 80(102028. https://doi.org/10.1016/j.npep.2020.102028

Liu Y, Li G, Wang X, Jia H, Dai J, Chen S and He D, 2025. Impact of fasting and refeeding on immune markers, hepatic gene expression, and gut microbiota in geese: insights into metabolic regulation and gut-liver interactions. Frontiers in Microbiology 16(1498460. https://doi.org/10.3389/fmicb.2025.1498460

Liu Y, Wang X, Li G, Chen S, Jia H, Dai J and He D, 2024. Investigating the impact of fasting and refeeding on blood biochemical indicators and transcriptional profiles in the hypothalamus and subcutaneous adipose tissue in geese. Animals 14(18): 2746. https://doi.org/10.3390/ani14182746

Luttinger D, King RA, Sheppard D, Strupp J, Nemeroff CB and Prange AJ, 1982. The effect of neurotensin on food consumption in the rat. European Journal of Pharmacology

81(3): 499-503. https://doi.org/10.1016/0014-2999(82)90116-9

Lutz TA, Tschudy S, Rushing PA and Scharrer E, 2000. Amylin receptors mediate the anorectic action of salmon calcitonin (sCT). Peptides 21(2): 233-238. https://doi.org/10.1016/s0196-9781(99)00208-9

Maier E and Brecht M, 2022. Maternity: Oxytocin circuits during birth and lactation. Current Biology 32(17): R921-R923. https://doi.org/10.1016/j.cub.2022.07.051

Maruyama T and Ueta Y, 2023. Internal and external modulation factors of the orexin system (REVIEW). Peptides 165: 171009. https://doi.org/10.1016/j.peptides.2023.171009

McKenzie B, Peloquin M, Tovar A, Graves JL, Ratcliff E, Tucker K, Vo K, Greenwood K, Halioua-Haubold CL and Juarez-Salinas D, 2024. Feeding dogs a high-fat diet induces metabolic changes similar to natural aging, including dyslipidemia, hyperinsulinemia, and peripheral insulin resistance. American Journal of Veterinary Research 85(6). https://doi.org/10.2460/ajvr.23.11.0253

Miller GD, 2019. Appetite regulation: hormones, peptides, and neurotransmitters and their role in obesity. American Journal of Lifestyle Medicine 13(6): 586-601. https://doi.org/10.1177/1559827617716376

Moran TH and McHugh PR, 1982. Cholecystokinin suppresses food intake by inhibiting gastric emptying. The American Journal of Physiology 242(5): R491-497. https://doi.org/10.1152/ajpregu.1982.242.5.R491

Nguyen TT, Kambe Y, Kurihara T, Nakamachi T, Shintani N, Hashimoto H and Miyata A, 2020. Pituitary adenylate cyclase-activating polypeptide in the ventromedial hypothalamus is responsible for food intake behavior by modulating the expression of agouti-related peptide in mice. Molecular Neurobiology 57(4): 2101-2114. https://doi.org/10.1007/s12035-019-01864-7

Olson BR, Drutarosky MD, Chow MS, Hruby VJ, Stricker EM and Verbalis JG, 1991. Oxytocin and an oxytocin agonist administered centrally decrease food intake in rats. Peptides 12(1): 113-118. https://doi.org/10.1016/0196-9781(91)90176-P

Payant MA, Shankhatheertha A and Chee MJ, 2025. Melanin-concentrating hormone promotes feeding through the lateral septum. Progress in Neuro-Psychopharmacology and Biological Psychiatry 136: 111163. https://doi.org/10.1016/j.pnpbp.2024.111163

Peng J, 2019. Gene redundancy and gene compensation: An updated view. Journal of Genetics and Genomics 46(7): 329-333. https://doi.org/10.1016/j.jgg.2019.07.001

Primeaux SD, Harrison-Bernard LM and Barnes MJ, 2021. Neurophysiology of the hypothalamus. In: Uwaifo, G.I. (Ed.), The Human Hypothalamus: Anatomy, Dysfunction and Disease Management. Springer International Publishing, Cham, pp. 33-52. https://doi.org/10.1007/978-3-030-62187-2_4

Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek J, Kanarek R and Maratos-Flier E, 1996. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380(6571): 243-247. https://doi.org/10.1038/380243a0

Raybould HE, 2007. Mechanisms of CCK signaling from gut to brain. Current Opinion in Pharmacology 7(6): 570-574. https://doi.org/10.1016/j.coph.2007.09.006

Rehfeld JF, 2025. Cholecystokinin: Clinical aspects of the new biology. Journal of Internal Medicine 298(3): 251-267. https://doi.org/10.1111/joim.20110

Roth JD, Erickson MR, Chen S and Parkes DG, 2012. GLP-1R and amylin agonism in metabolic disease: complementary mechanisms and future opportunities. British Journal of Pharmacology 166(1): 121-136. https://doi.org/10.1111/j.1476-5381.2011.01537.x

Rouf MA, Wen L, Mahendra Y, Wang J, Zhang K, Liang S, Wang Y, Li Z, Wang Y and Wang G, 2023. The recent advances and future perspectives of genetic compensation studies in the zebrafish model. Genes & Diseases 10(2): 468-479. https://doi.org/10.1016/j.gendis.2021.12.003

Sahu A, Carraway RE and Wang YP, 2001. Evidence that neurotensin mediates the central effect of leptin on food intake in rat. Brain Research 888(2): 343-347. https://doi.org/10.1016/S0006-8993(00)03107-3

Saito Y, Nothacker HP, Wang Z, Lin SH, Leslie F and Civelli O, 1999. Molecular characterization of the melanin-concentrating-hormone receptor. Nature 400(6741): 265-269. https://doi.org/10.1038/22321

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richarson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan R S, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ and Yanagisawa M, 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92(5): 697. https://doi.org/10.1016/s0092-8674(02)09256-5

Schwartz MW, Woods SC, Porte DJ, Seeley RJ and Baskin DG, 2000. Central nervous system control of food intake. Nature 404(6778): 661-671. https://doi.org/10.1038/35007534

Song J and Choi SY, 2023. Arcuate nucleus of the hypothalamus: anatomy, physiology, and diseases. Experimental Neurobiology 32(6): 371-386. https://doi.org/10.5607/en23040

Sureshkumar K, Saenz A, Ahmad SM and Lutfy K, 2021. The PACAP/PAC1 receptor system and feeding. Brain Sciences 12(1). https://doi.org/10.3390/brainsci12010013

Tran LT, Park S, Kim SK, Lee JS, Kim KW and Kwon O, 2022. Hypothalamic control of energy expenditure and thermogenesis. Experimental & Molecular Medicine 54(4): 358-369. https://doi.org/10.1038/s12276-022-00741-z

Tsujino N and Sakurai T, 2013. Role of orexin in modulating arousal, feeding, and motivation. Frontiers in Behavioral Neuroscience 7(28. https://doi.org/10.3389/fnbeh.2013.00028

Volčanšek Š, Koceva A, Jensterle M, Janež A and Muzurović E, 2025. Amylin: From mode of action to future clinical potential in diabetes and obesity. Diabetes Therapy 16(6): 1207-1227. https://doi.org/10.1007/s13300-025-01733-8

Vu JP, Luong L, Sanford D, Oh S, Kuc A, Pisegna R, Lewis M, Pisegna JR and Germano PM, 2023. PACAP and VIP neuropeptides' and receptors' effects on appetite, satiety and metabolism. Biology 12(7). https://doi.org/10.3390/biology12071013

Walker CS, Aitken JF, Vazhoor Amarsingh G, Zhang S and Cooper GJS, 2025. Amylin: emergent therapeutic opportunities in overweight, obesity and diabetes mellitus. Nature Reviews Endocrinology 21(8): 482-494. https://doi.org/10.1038/s41574-025-01125-9

Weng L, Zhang J, Peng J, Ru M, Liang H, Wei Q, Ruan J, Ali R, Yin C and Huang J, 2025. Functional remodeling of gut microbiota and liver in laying hens as affected by fasting and refeeding after fasting. Animal Bioscience 38(4): 692-706. https://doi.org/10.5713/ab.24.0299

Yukinaga H and Miyamichi K, 2025. Oxytocin and neuroscience of lactation: Insights from the molecular genetic approach. Neuroscience Research 216(104873. https://doi.org/10.1016/j.neures.2025.01.002