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Short Commentary

Impact of Insulin on Memory Recall

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Abstract

Recent studies revealed that an injection of insulin into the central nervous system ameliorates deficits in memory formation and recall in snails. A spontaneous increase in the insulin levels in snails is also associated with improved memory recall. Thus, insulin is thought to be a critical factor for memory recall in snails. In the present study, we describe the production and function of insulin in the snail central nervous system for memory recall and expand this scenario to other animals. These findings provide a new avenue for studying the mechanisms underlying learning and memory.

Keywords: Conditioned taste aversion; Insulin; *Lymnaea*; Memory recall; Snail

INTRODUCTION

In 1988, a mini-review titled "Invertebrate neuroendocrinology. Insulin found at last?" in Nature astounded researchers in the field of invertebrate neuroscience [1]. This review highlighted the article by Smit and colleagues who found molluscan insulin-related peptides (MIPs) in the central nervous system (CNS) of the pond snail *Lymnaea stagnalis* [2]. *Lymnaea* MIPs were the first established insulin-like peptides in invertebrates, and since then Smit and colleagues have clarified various aspects of MIPs, such as their involvement in growth control and egg-laying [3]. Eighteen years later, Azami et al. provided evidence demonstrating that MIPs are involved in learning and memory in *Lymnaea* [4]. They used the conditioned taste aversion (CTA) protocol, in which a sucrose solution is used as the conditioned stimulus (CS) and a KCl solution is used as the unconditioned stimulus (US) [5,6]. Application of the CS to the lips increases the feeding response, whereas application of the US inhibits it. After repeated temporal pairings of the CS and US, the CS no longer elicits feeding [5]. Using a cDNA microarray system, Azami and colleagues found that MIP gene expression is upregulated during CTA formation [4].

The CTA response persists for at least a month [5], and is classified as long-term memory (LTM) according to the temporal classification of memory [6]. LTM should include a de novo protein-synthesis process [7], and a protein synthesis-dependent period during the consolidation of *Lymnaea* CTA to LTM has been demonstrated by pharmacologic inhibition of transcription or translation [8]. The robustness for CTA-LTM was confirmed by extinction trials, because repeated presentations of the CS alone after establishing a CTA fails to extinguish the CTA. Furthermore, the CTA is formed even when the presentation of the US is delayed. Thus, CTA in *Lymnaea* is thought to be similar to CTA in mammals.

In the present study, we review the production of insulin, MIPs, in the snail CNS and then describe the effects of insulin on memory formation and memory recall. Finally, we expand the scenario of the involvement of insulin in learning and memory to other animals.

MIPs IN THE SNAIL CNS

Five types of MIPs, MIP I, II, III, V, and VII, are produced in *Lymnaea* [9-14]. The neurons that synthesize MIPs are the growth controlling neuroendocrine light green cells (LGCs) and canopy cells in the snail CNS [15-17]. *In situ* hybridization experiments for MIP II, the major MIP, clarified that the signals are localized in the LGCs [18]. On the other hand, only one MIP receptor gene is reported for these different MIPs [16]. The cDNA structure of this MIP receptor is a putative tyrosine kinase receptor [19], and *in situ* hybridization signals for the MIP receptor are observed in many neurons throughout the CNS.

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Murakami and colleagues demonstrated that applying MIPs to an isolated *Lymnaea* CNS preparation evokes long-lasting synaptic enhancement at synapses involved in the feeding response [18]. This synaptic enhancement is blocked by simultaneous application of an anti-insulin receptor antibody. They used a human, not snail, antibody because there was evidence that the human antibody would block the binding between MIP and the MIP receptor (for a detailed explanation, see the Murakami study). Direct injection of this anti-insulin receptor antibody into the snail body also blocks LTM formation at the behavioral level. Because *Lymnaea* has an open blood-vascular system, this blockade is thought to occur in the CTA-related neural circuits in the CNS.

MIPs FOR MEMORY RECALL IN SNAIL

A relation between the food-deprivation state and memory recall for CTA is observed in *Lymnaea* [20-23]. Mildly food-deprived snails (i.e., 1-day food deprivation) can form a strong CTA, whereas heavily food-deprived snails (i.e., 5-day food deprivation) do not seem to form a CTA. When the heavily food-deprived snails are injected with insulin, however, they, too, exhibit CTA-LTM [21-24]. This effect of insulin for attenuating the deficiency of CTA-LTM in heavily food-deprived snails can be abolished by simultaneously injecting the anti-insulin receptor antibody described above [21-24].

From the above results, we hypothesized that the heavily food-deprived snails form a CTA, but do not exhibit memory recall, because very hungry snails eat whatever is in front of them, as if 'necessity knows no law' [25,26]. The results obtained support this hypothesis. Moreover, the effect of insulin injection on memory recall in the heavily food-deprived snails, as described above, was mimicked by mild food deprivation as described below. After snails were heavily food-deprived (e.g., 5 days), CTA training was performed. At this point, the snails did not demonstrate CTA-LTM. The snails were then provided ad libitum access to food for a few days to keep them healthy. Subsequently, the snails were mildly food-deprived (e.g., 1 day). These snails exhibited good memory recall [25,26]. We therefore concluded that the optimal internal state for memory recall of CTA is provided by either an injection of insulin or mild food deprivation.

More recently, Totani et al. observed a difference in MIP II mRNA expression levels in the snail CNS, as described above, using real-time polymerase chain reaction (PCR) experiments [27]. The study comprised 3 cohorts of snails. (A) Snails that were heavily food-deprived, trained with the CTA training protocol, and then provided food. These snails were referred to as snails without mild food deprivation. (B) Snails that were heavily food-deprived, trained with the CTA training protocol, provided food, and then mildly food-deprived. These snails exhibited good memory recall and were referred to as the good memory-recall performers (defined as a snail that made 0 - 1 bites/min during the memory recall test in response to presentation of the CS [20]). (C) Snails that were heavily food-deprived, trained with the CTA training protocol, provided food, and then mildly food-deprived. These snails exhibited poor memory recall and were referred to as the poor memory-recall performers (defined as a snail that made ≥ 2 bites/min in response to the CS during the memory recall test).

Totani et al. found that MIP II levels were significantly higher in the good memory-recall performers after mild food deprivation, compared with the poor performers and the snails without mild food deprivation (Figure 1).

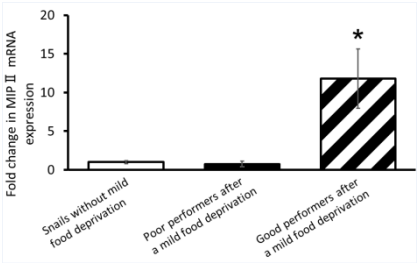

Figure 1: Comparison of MIP II mRNA levels among snails without mild food deprivation, poor memory-recall performers, and good memory-recall performers after mild food deprivation. * P<0.05. These data show that in snails exhibiting good memory recall, the MIP II level increases during mild food deprivation. The definition of good and poor performers is provided in the text. The original data were published in Totani et al., 2020 [27], and re-use was permitted under the terms of the Creative Commons Attribution 4.0 International license.

That is, the level of MIP II, the major MIP, spontaneously changes and increases during mild food deprivation. Therefore, the effects of insulin injection are similar to those of mild food deprivation in *Lymnaea* CTA [27].

EXPANSION OF THE INSULIN SCENARIO FOR LEARNING AND MEMORY TO OTHER ANIMALS

The most popular studies of the contribution of insulin function to cognition in humans involves a treatment for Alzheimer disease [28]. Clinical studies reveal that intranasal insulin administration provides reliable treatment results in patients with Alzheimer disease. Many studies have evaluated insulin function in invertebrates, such as *Caenorhabditis elegans* and *Drosophila* [29,30]. Especially in Drosophila, the relation between the actions of insulin and those of cAMP-regulated transcriptional coactivator (CRTC) has been clarified [31]. CRTC is thought to be activated when the insulin signaling cascade is downregulated in hungry flies [32]. Hungry flies learn conditioned food aversions by 1-trial learning because of the downregulation of the insulin signaling pathway and the upregulation of the CRTC signaling pathway [33]. Because the state of hunger in flies is caused by food deprivation for 9 to 16 h before conditioned food aversion, it may correspond to the state of mild food deprivation in our *Lymnaea* studies. The relation between the insulin signals and the CRTC signals in *Lymnaea* has not yet been studied, and thus the molecular mechanisms should be carefully examined in *Lymnaea*.

CONCLUSION

The involvement of insulin function in learning and memory is not restricted to snails, but may occur across phyla. For a comprehensive understanding of the involvement of insulin in

learning and memory, subsequent studies should target the cascades from insulin reception to a de novo protein synthesis mechanism. The transcription factors involved in protein synthesis may include cAMP-responsive element binding protein, which is well analyzed in *Lymnaea*. We hope that this clarification of insulin function in learning and memory opens up new approaches for studying the mechanisms underlying learning and memory.

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CONFLICT OF INTEREST

None to report.

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