HYDRA AS AN ANIMAL MODEL SYSTEM IN PHYSIOLOGY

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ABSTRACT
Hydra has been used as a model in different topics in biology, including physiology. It pertains to the Phylum Cnidaria, an ancestral group of Metazoa that shares a common ancestor with Bilateria. Hydra provides an experimental framework to analyze mechanisms that regulate the homeostasis and, due to the high level of gene conservation, can be easily extrapolated to other animal groups. We use this model to analyze physiological and evolutionary aspects of communication systems activated by food, and by the peptide messengers, Allatotropin (AT) and Allatostatin-C (AST-C). Using immunohistochemistry, quantum dots, bioinformatic, and physiological assays, we show that these systems are present in Hydra regulating feeding behavior. We analyze the regulation of the extrusion of the hypostome (that contains the mouth), showing that, whereas AT mimics the effect of the food, AST-C acts as antagonist. We show that those effects depend on changes in the cytosolic Ca^{2+} concentration, revealing the specific signaling pathway activated. Our data support the ancestral functions and conservation of these signaling systems that control myoregulatory activities related with feeding.
Keywords: Hydra; myoregulation; model system; physiology.

RESUMEN
Hydra ha sido usada como modelo para el estudio de diferentes aspectos de la biología, incluyendo fisiología. Pertenece al phylum Cnidaria, grupo ancestral de metazoos que comparte un ancestro común con Bilateria. El modelo de Hydra representa un sistema de estudio útil para analizar el mantenimiento de la homeostasis que, debido al alto grado de conservación respecto de los metazoos, puede ser extrapolado a otros grupos animales. Analizamos en Hydra tanto aspectos fisiológicos, como evolutivos de sistemas de comunicación activados por el alimento y los péptidos allatotropina(AT) y allatostatina C (AST-C). Mediante análisis de datos inmunohistoquímicos, *quantum dots*, fisiológicos y de bioinformática demostramos que estos sistemas de comunicación están presentes en Hydra regulando el comportamiento alimentario. analizando la regulación de la extrusión del hipostoma (estructura que contiene la boca) demostramos que AT mimetiza el efecto del alimento, estimulando su extrusión, mientras que AST-C se comporta como antagosnista. Mostramos que estos efectos dependen de cambios en la concentración de Ca^{+2} citosólico, revelando sus vías de señalización especifica. Nuestros datos muestran las funciones y conservación de estos sistemas de comunicación, desde organismos ancestrales como Cnidarios.
Palabras clave: Hydra; mioregulación; sistema modelo; fisiología.

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Introduction

Hydra is a fresh-water member of the phylum Cnidaria, a basal group of metazoans. This phylum evolved 700 million years ago, and represents the first group of animals with a defined body axis and a nervous system [1]. Together with Ctenophora and Placozoa, it shares a common ancestor with Bilateria (http://tolweb.org/Animals/2374) (Figure 1D).

The anatomy of Hydra is simple (Figures 1A and B). It has a tubular body, with differentiated structures along their axis performing specific functions. The oral region is at the apical pole, and is responsible for the active feeding behavior. This region consists in a ring of tentacles (that participates in the prey capture) surrounding the hypostome, that contains the mouth. At the opposite pole (aboral) the body presents the basal disc, responsible for the adhesion to the substrate. The digestive system is incomplete, constituted by a gastrovascular cavity (GVC), in which the digestion takes place. This cavity opens at the oral pole in the mouth/anus area. The nervous system consists of a net of neurons (sensory-motor, ganglia, and mechanosensory) along the body column. This net shows a higher density at both extremities, and forms a nerve ring just around the tentacles. It provides robust neuro-muscular activities with sophisticated behaviors. The reproduction is mainly asexual. Indeed, when the food is abundant the body generates buds, that after an appropriated growth separate from their parents. In some conditions, the individuals can develop gonads to reproduce sexually.

The body wall consists of two myoepithelial layers separated by an extracellular matrix, called mesoglea (Figure 1C). The outer layer (ectoderm) consists of multifunctional myoepithelial cells. In the inner layer (endoderm) the myoepithelial cells also act as digestive cells, phagocytosing nutrients together with a population of gland cells that secrete proteases [2].

Myoepithelial cells may represent the most ancestral type of contractile cells, and exhibit the eumetazoan actin-myosin machinery [3]. They present contractile projections that are highly organized, running axially along the body in the ectoderm, and circumferentially in the endodermic layer. In this sense, contraction of ectodermal myoepithelial cells results in a decrease of the length of the body column, whereas the contraction of the cells in the endoderm produces the lengthening of these structures [4, 5]. These contractile cells are innervated, with synaptic structures that that resembles the neuromuscular junctions of the bilaterians [3].

Beyond myoepithelial and secretory cell populations, both layers also contain nerve cells, interstitial cells, and when appropriate, gametes. Moreover, Hydra sp. as a member of the phylum Cnidaria, present another particular and characteristic cell population, the nematocytes, which are a kind of mechano-sensory cells related with prey capture that constitute a synapomorphy of this phylum.

Finally, these cell lineages (i.e. myoepithelial and interstitial cells), behave as stem cells for a dozen of cell types [6].

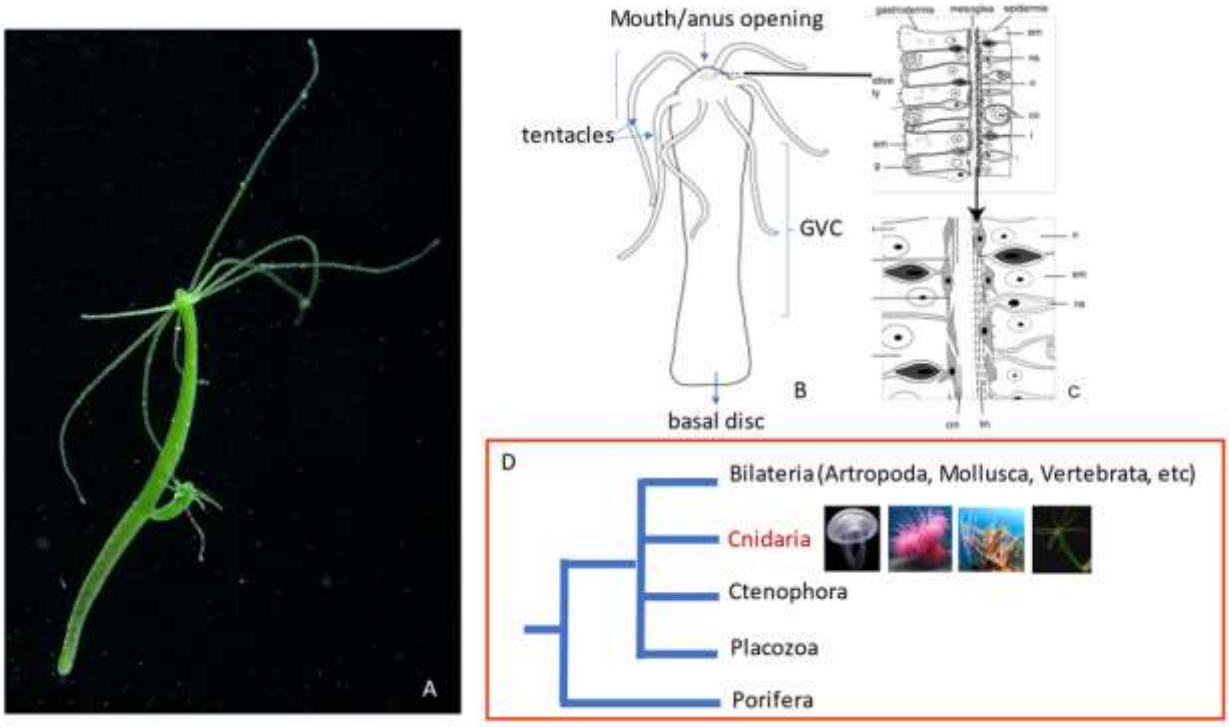

Figure 1. Anatomy of Hydra. A. General aspect of Hydra sp. B. Schematic representation of Hydra sp with the main regions of the body. GVC: gastrovascular cavity C. Cross sections of the hydroid body showing the two body layers (ectoderm or epidermis and endoderm or gastrodermis) and the mesoglea between them. The scheme also shows the different cells that form the body walls. cn: cnidocyte; eg: epithelial gland cell; ep: epithelial-muscular cell; g: mucous and enzymatic gland cell; i: interstitial cell; n: nerve cell and ns: neurosensory cell; cm: circular muscular layer formed by the contractile extensions of the nutritive muscle cells; lm: longitudinal muscle layer formed by the contractile extensions of the epithelial-muscular cells. (Modified of Alzugaray et al., 2013) D. Phylogenetical position of the Phylum Cnidaria showing its relationships with other groups of metazoans.

Hydra as an animal model system

Due to its great capacity of asexual reproduction and regeneration, Hydra colonies are easy to maintain in the laboratory. For many years different species were used as an animal model system to study different aspects of biology, including physiology, ecosystems, ecotoxicology, and developmental biology [reviewed in 7]. Regarding this last topic, Hydra is probably the most well-known cnidarian because of its low senescence rate, and its regenerative capabilities, being practically immortal. Indeed, Hydra polyps not only can regenerate any lost part of its body, but also, can reaggregate into an intact animal within a few days after being dissociated into single cells [8].

Hydra in physiological studies

Regarding physiology, since the late 19th century, Hydra became a model system because it provides an experimental framework to analyze mechanisms that regulate the maintenance of homeostasis that can be easily extrapolated to other animal groups. In this way, one of the most studied aspect has been its active feeding behavior, by which the prey is captured and ingested in a complex and coordinated fashion, when signals from their preys initiate this response.

Analyzing the feeding behavior in Hydra: effects of AT and AST-C peptides

Our laboratory has studied communication systems that involve the peptidic messengers Allatotropin (AT) and Allatostatin-C (AST-C). These signaling molecules were first described in insects by their function as regulators of the synthesis and secretion of juvenile hormones (JH) [9, 10]. They exert their functions by binding to membrane receptors of the rhodopsin subfamily of G protein-coupled receptors (GPCRs), that are considered orthologues of the orexin (Ox) and somatostatin (STT) receptors of Chordata, respectively [11, 12-15].

Both peptides have many other roles, being the regulation of muscle contraction one of the most well documented. Whereas AT has myo and cardiostimulatory effects, AST-C shows an inhibitory effect, acting as an antagonist of the myostimulatory activity of AT [16-18]. Although these peptides and their receptors were mainly studied in insects, the existence of AT and AST-C-like systems has been proposed in other phyla of invertebrates as Annelida, Mollusca, Platyhelminthes, and Cnidaria [19-21,13].

In our laboratory, we use the Hydra model to study not only the physiological aspects of this organism, but also to analyze communication systems based in AT and AST-C, in terms of the evolution in Metazoa. Using AT and AST-C peptides conjugated with nano-crystals (quantum dots), we showed that these peptides are recognized by different myoepithelial cell populations in Hydra sp., suggesting that Hydra cells can respond to these signaling molecules due to the existence of specific receptors for both peptides [13, 21].

Physiological assays showed that both, AT and AST-C act as myoregulators in Hydra, inducing different behaviors. The treatment with AT causes the extrusion of the hypostome, mimicking the effect of food during feeding behavior. Moreover, the treatment with an AT antiserum avoids hypostome extrusion induced by the presence of food [14, 21]. Regarding AST-C treatment, it induces a significant change in the length and shape of tentacles, mimicking those adopted during the capture of the prey. AST-C also causes a shortening of the body column, mainly by the shortening of the peduncle. Finally, the gastrovascular cavity (GVC) changes to a bottle like shape, resembling the appearance adopted after ingestion of the prey. When the maximum dose of this peptide was used, it triggered contractions which mimicked peristaltic waves [13]. Interestingly, this peptide did not show any relevant effect on the hypostome of starved hydroids, but prevented its extrusion when was applied on hydroids stimulated with food or AT [14]. Altogether these results suggest that AT and AST-C could participates in the regulation of the feeding behavior in Hydra.

Results obtained by the use of bioinformatic tools support those obtained by physiological assays. In spite of searches for AT and AST-C peptides in cnidarians genomes did not show any relevant sequences to be considered as orthologue of these neuropeptides, in silico searches for the receptors do show that Cnidaria genomes predict the existence of GPCRs that would be orthologues of the systems that are present in bilaterians. Regarding AST-C, we found proteins that show a high level of identity with the family of AST-C/Somatostatin receptors. Interestingly, the putative proteins from cnidarians show a high level of identity and similitude in the sequences considered as a signature of this family [13]. Proteins sharing homology with AT/Ox receptor also were predicted by cnidarian genomes [21,14,15]. Indeed, they have the E/DRWYAI motif, which is considered as a signature of this family of receptors [15].

Study of signaling pathways in Hydra: understanding the feeding behavior

Most of the studies performed in Hydra analyze pathways involved in the control of development and aging. However, basic physiological mechanisms such as muscle contractions, remains poorly understood.

An increase in the cytosolic calcium levels ([Ca2+]cyt) is needed to induce muscle contraction in any type of muscle cells. The pioneering studies of feeding behavior developed in Hydra sp., shows that EDTA (a Ca2+ chelator that acts in the extracellular medium) inhibits feeding, and also that the addition of Ca2+ reverses this effect [22, 23]. On the other hand, studies performed in muscles of jellyfish (Cnidaria), show that an increase in [Ca2+]cyt is due to both, the release of Ca2+ from intracellular stores, and its influx from the extracellular space [24, 25]. To understand the involvement of this ion in the extrusion of the hypostome in Hydra, we analyzed its behavior in the presence of different compounds that modify Ca2+ levels, and stimulates or inhibit the cellular Ca2+ machinery.

Hypostome extrusion induced by food and AT peptide depends on an increase of [Ca2+]cyt

Treatments of starved hydroids with thapsigargin (TG), a compound that inhibits the reuptake of Ca2+ into the sarco(endo)plasmic reticulum (ER) inducing an increment of its cytosolic levels, causes an increase in the length of the hypostome, similarly to the presence of both, food and AT. In agreement with these results, treatments with the intracellular Ca2+ chelator BAPTA/AM, prevent the hypostome extrusion induced by food or AT in a dose-dependent fashion. These facts suggest that an increase in the [Ca2+]cyt is required for hypostome activity, and that one of the ways involved is the release of Ca2+ from de ER [14, 26, 27]. Similar results were obtained in physiological assays using EDTA (a Ca2+ chelator) or nifedipine, a blocker of voltage-gated Ca2+ channels (VGCC), which prevent the influx from the extracellular media. In both assays the treatment avoided the hypostome extrusion. Altogether these results suggest that in Hydra, both mechanisms that increase [Ca2+] cyt (i.e. release ER, and extracellular influx throughout an L-Type VGCC) are relevant for hypostome extrusion [14]. Interestingly, EDTA did not completely prevent the response induced by food, suggesting that the influx of calcium is not the only way involved to cause the extrusion of the hypostome. The release of Ca2+ from the ER (the main intracellular source of Ca2+ in muscle cells) is mediated by two types of channels: Ryanodine receptors (RYR), which are primarily modulated by Ca2+, and inositol-3 phosphate receptors (IP3R) which are activated by different ligands that bind with cell membrane receptors which produce IP3. Using physiological experiments, we also tested the involvement of both ER Ca2+ receptor-channels in Hydra and found that treatments with Xetospongine-C (Xe-C) (an inhibitor of IP3R) prevents the extrusion of the hypostome induced by food or AT, while treatments of starved hydroids with caffeine (an agonist that causes the opening of the RyR) increase the hypostome length, mimicking the effect of the stimulators of the feeding behavior. Moreover, assays using inhibitory doses of ryanodine (Ry) (a vegetal compound that blocks the RyR) prevented hypostome extrusion in the presence of food but has no effect in hydroids treated with AT. Taking together, these results suggest that the two known ways to release Ca2+ from ER participate in the hypostome extrusion induced by food, but the IP3R is the one involved in the mechanism activated by AT [26, 27]. Using bioinformatic tools we found that H. vulgaris genome predicts a protein that fits all of the characteristics of an IP3R. Although experimental results suggest the existence of proteins reacting to Ry and caffeine, RyR orthologues were not found in this Phylum [26]. In agreement with our finding, other authors have also suggested that RyR would not be present in Cnidaria [28]. The existence of RyRs has been predicted in many other groups of invertebrates, as well as in the basal metazoan Trichoplax adhaerens (Placozoa) that lacks neurons and muscles, and it also is present in unicellular eukaryotes such as choanoflagellates, suggesting that RyR evolved early in evolution, but could have been lost in certain metazoan groups such as Cnidaria [28]. Regarding our results, it is possible that other kind of channel proteins could be acting as the Ry target in Cnidaria [26].

Hydra as a model to study GPCR and their signaling cascades

As we mentioned previously, AT acts by binding to a receptor of the GPCR superfamily. In spite that the receptor has been widely studied in insects, the signaling cascade is not completely understood. We use the Hydra model, and the extrusion of the hypostome, to analyzed the complete signaling pathway activated by AT peptide in vivo [27].

Taking into account that our previous results suggest the involvement of the IP3R in the mechanism activated by AT in Hydra, and due that IP3Rs open when they have bound the second messenger IP3, we analyzed this signaling cascade in Hydra. IP3 is produced by the cleavage of a membrane phospholipid, the PI2P by the enzyme phospholipase C (PLC). PLCs can be grouped into six subfamilies (i.e. β, γ, δ, ε, ζ, η), which are activated by different mechanisms, including GPCRs. In the lack of a subtype-selective PLC inhibitor, we used U73122, a compound that inhibits all PLC types. The treatment with this compound prevents the effect of AT peptide on hypostome extrusion. Altogether, the inhibition of PLC and IP3R, confirm the involvement of the IP3 pathway in the transduction of AT signaling.

In order to confirm the involvement of a GPCR in this signaling pathway, we treated hydroids with an inhibitor of agonist and antagonist binding to GPCRs (SCH-202676). The results showed that SCH completely inhibited hypostome extrusion induced by AT, providing supporting evidence on the involvement of this kind of receptor in the signal transduction of this peptide, and suggesting that ATR would be linked to a member of the Gαq subfamily (i.e. Gαq, Gα11, Gα14 or Gα15/16), which activates the β subfamily of PLCs [27]. Indeed, studies performed in Porifera and Cnidaria showed that genes codifying proteins involved in the inositol phospholipid signaling pathway, such as Gαq and PLCs (including the β subtype), would appeared early in the evolution of Metazoa, suggesting the ancestral origin in the earliest branching of animal phyla. [29].

Another possible pathway related to GPCRs, is the one that controls the levels of cAMP throughout the regulation of the activity of the enzyme adenylyl cyclase (AC). This enzyme converts ATP into cAMP. While Gαs subfamily of G protein stimulates AC, Gαi inhibits it. We also analyzed the involvement of this pathway in our experimental model. The results obtained in hydroids undergoing treatment with melittin, a compound that inhibits Gαs and stimulates Gαi activity, suggest that this pathway is not relevant for the hypostome extrusion in Hydra.

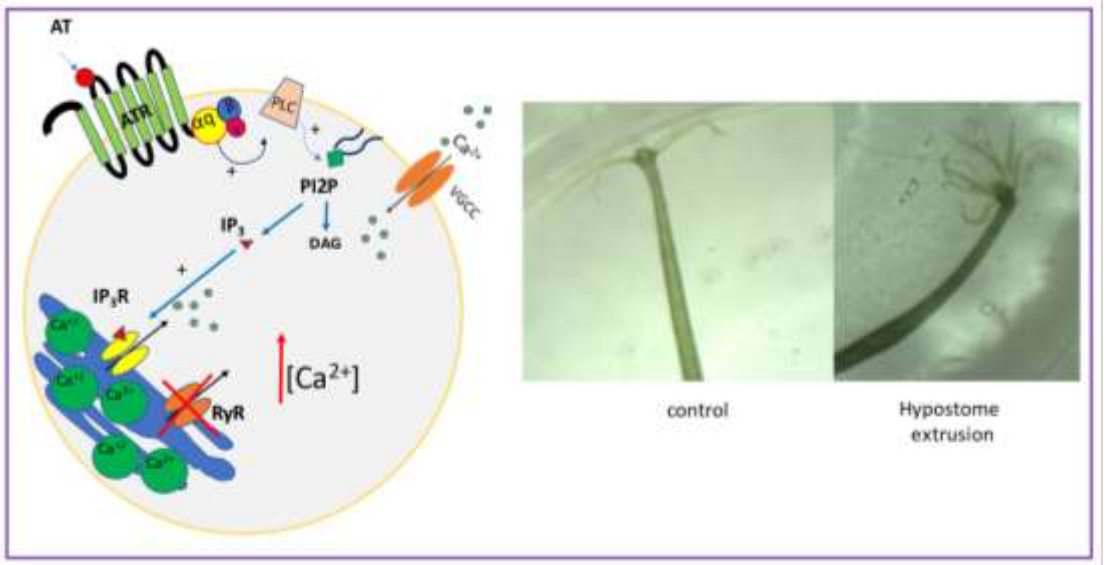

Figure 2. Signaling pathway of AT in Hydra sp activated during hypostome extrusion. Schematic representation of the signaling cascade in epithelia-muscle cells. VGCC: voltage-gated Ca2+ channels; ATR: Allatotropin receptor (GPCR); αβγ: subunits of the G protein; αq: subtype q of the G protein; PLC: phospholipase; PI2P: phosphatidyl inositol bi phosphate; DAG: diacyl-glycerol; IP3: inositol-tri-phosphate; IP3R: inositol-tri-phosphate receptor; RyR: ryanodine receptor.

Interestingly, the ATR signaling pathway proposed in our model resembles those ones activated by OxRs in vertebrates, which in fact are phylogenetically related [11, 14, 15] In spite of OxR signaling is complicated and versatile (for review see 30), both orexin receptors can be coupled to Gαq proteins, inducing PLC activity and Ca2+ release from ER. Even though Ox peptides regulate complex functions, they also act as myoregulators. In fact, it was shown that they modulate intestinal and cardiac muscle contraction [31, 32]. In fact, in intestinal smooth muscle, it was inhibited by the L-type VGCC blocker nifedipine, and by inhibitors of the IP3R [31]. This data supports the ancestral existence of the protein machinery involved, and the pathways that control myoregulatory activities related with feeding. Indeed, Hydra sp was the first species in which the conservation of molecules regulating cell differentiation and development across the biological evolution was demonstrated. Interestingly, some of these molecules show identical sequences between cnidarians and mammals. [33]. A trasncriptomic analysis showed that Hydra sp shares at least 6071 genes with humans, in contrast to Drosophila sp and Caenorhabditis elegans, which share only 5696 and 4571 genes, respectively, with humans [34]. Furthermore, when the genome of Hydra magnipapilata was available [3] it was confirmed that most of the gene families present in mammals, do have representatives in cnidarians.

Conclusions

For more than 250 years Hydra has been used as a model in different topics of biology. In addition to their high rate of reproduction and regeneration, this group of species also has the advantage of show a high level of gene conservation in comparison with more recent animal groups, that strengthens the validity of this model systems for studying processes shared by eumetazoans. In our laboratory, we use the Hydra model to study, not only the physiological aspects of this organism, but also to analyze processes in terms of the evolution of Metazoa.

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