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Somatostatin signaling system as an ancestral mechanism: Myoregulatory activity of an Allatostatin-C peptide in Hydra

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ABSTRACT

The coordination of physiological processes requires precise communication between cells. Cellular interactions allow cells to be functionally related, facilitating the maintaining of homeostasis. Neuropeptides functioning as intercellular signals are widely distributed in Metazoa. It is assumed that neuropeptides were the first intercellular transmitters, appearing early during the evolution. In Cnidarians, neuropeptides are mainly involved in neurotransmission, acting directly or indirectly on epithelial muscle cells, and thereby controlling coordinated movements. Allatostatins are a group of chemically unrelated neuropeptides that were originally characterized based on their ability to inhibit juvenil hormone synthesis in insects. Allatostatin-C has pleiotropic functions, acting as myoregulator in several insects. In these studies, we analyzed the myoregulatory effect of *Aedes aegypti* Allatostatin-C in *Hydra* sp., a member of the phylum Cnidaria. Allatostatin-C peptide conjugated with Qdots revealed specifically distributed cell populations that respond to the peptide in different regions of hydroids. *In vivo* physiological assays using Allatostatin-C showed that the peptide induced changes in shape and length in tentacles, peduncle and gastrovascular cavity. The observed changes were dose and time dependent suggesting the physiological nature of the response. Furthermore, at highest doses, Allatostatin-C induced peristaltic movements of the gastrovascular cavity resembling those that occur during feeding. *In silico* search of putative Allatostatin-C receptors in Cnidaria showed that genomes predict the existence of proteins of the somatostatin/Allatostatin-C receptors family. Altogether, these results suggest that Allatostatin-C has myoregulatory activity in *Hydra* sp, playing a role in the control of coordinated movements during feeding, indicating that Allatostatin-C/Somatostatin based signaling might be an ancestral mechanism.

1. Introduction

The coordination of physiological mechanisms requires the communication between cells in all organisms. By these cellular interactions, tissues and organs are functionally related allowing the organisms to accomplish integrated functions maintaining homeostasis. Peptidic molecules functioning as signals for intercellular communication are widely distributed in Metazoa, playing important regulatory roles in a variety of physiological processes. These molecules are mainly pleiotropic, acting as neurotransmitters and neuromodulators in the nervous system, and as hormones in endocrine and neuroendocrine systems. In fact, it has been proposed that neuropeptides were the first intercellular transmitters, being present early during the evolution of the nervous-system [14].

Although neuron-like cells have not been described in Placozoa, the existence of neurosecretory-like cell populations [32], and cells immunoreactive to neuropeptides [29] have been proposed. Placozoa and Cnidaria are among the most basal metazoan phyla. Furthermore, it was proposed that Cnidaria is the first group of living organisms with a developed nervous system [15], and it is assumed that it originated from the common ancestor of protostomia and deuterostomia [36].

Signaling mechanism based on peptides depends on the presence of receptors located on the target cell surface. In most cases, the molecules receiving the signal for these peptidic ligands, are members of the ubiquitous, largest and most versatile family of receptors, the G protein-coupled receptors (GPCRs), which are

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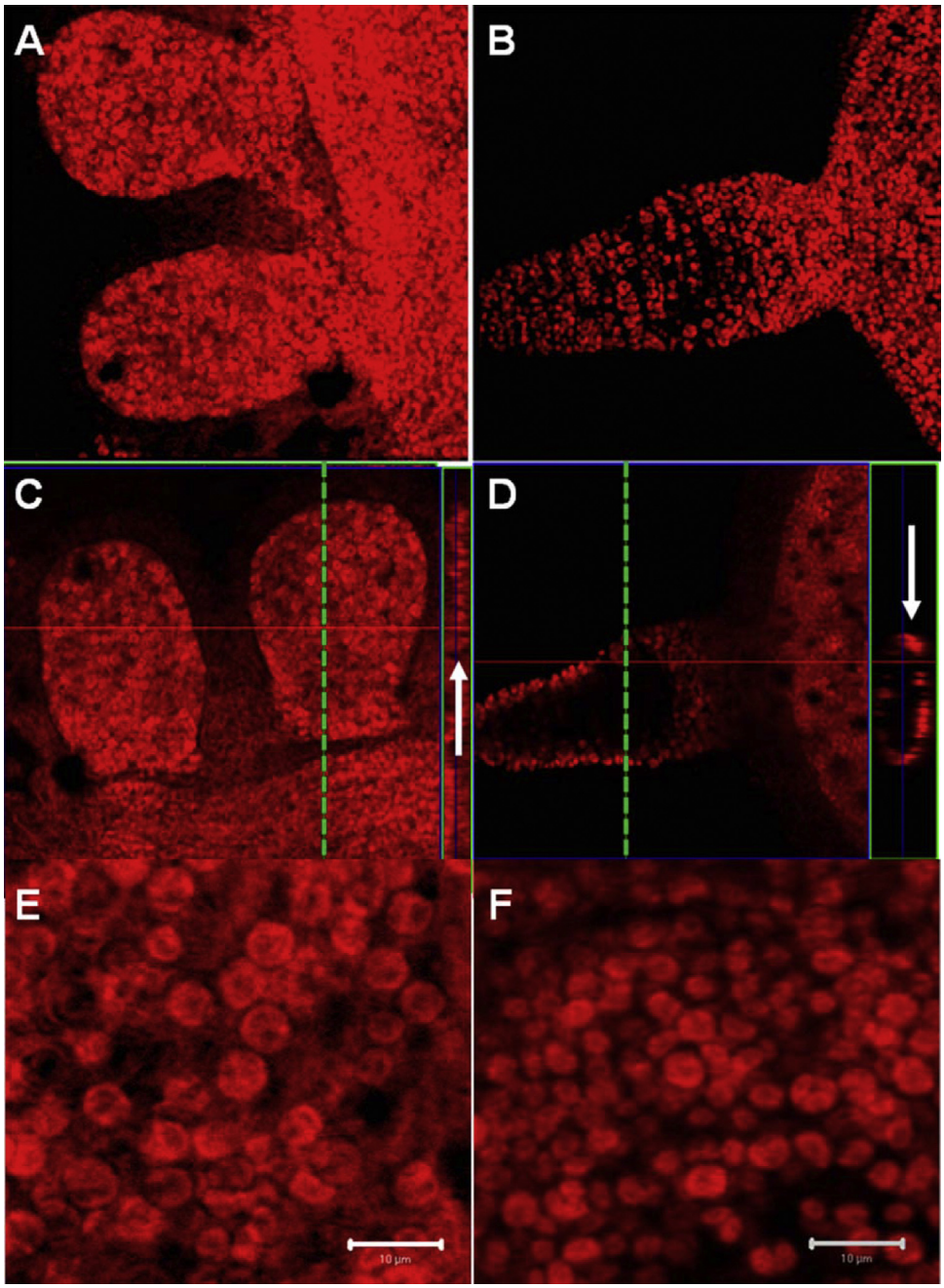

Fig. 1. Qdot-AT and AST-C labeling. View of the tentacles showing the specific distribution of cells labeled with AST-C (A) and AT (B). Note that the overall distribution of the cells is different. (C and D) Orthogonal sections of AST-C and AT labeled tentacles showing the distribution of labeled cells. The Qdot-AST-C labeled cells are located in the inner layer of the tentacles (C) whilst the AT recognizing cells are located in the outer layer. Green dashed lines indicate the plane of the optical cut. Arrows show orthogonal sections of the tentacles. Note that the tentacle in (D) looks as an empty structure. (E and F) Magnified views showing the cells of the tentacles recognizing AST-C (E) and AT (F). The different distribution and size of the labeled cells corresponding to both peptides is evident. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

characterized for the presence of 7 transmembrane domains (TM) and the existence of characteristic sites determining conformational changes to activate G protein [25,41]. This signaling pathway is already present in unicellular eukaryotes such as yeasts [11], in basal metazoans such as *Trichoplax adhaerens* (Placozoa) and *Hydra* sp. (Cnidaria) [1,3]; and in fact they are highly conserved in all metazoan [13].

Hydra sp. has a simple body plan that consists in a columnar body divided into a distal region that contains the mouth and tentacles, a proximal region containing the peduncle and the basal disk, and a gastric region between them. The body of the hydroid consists in a few cell types organized in two layers, ectoderm and endoderm, separated by a non-cellular layer, the mesoglea. In both tissue layers there are unique epithelial muscle cells [9] that exhibit the eumetazoan actin-myosin machinery. The contractile projections of these cells are highly organized running axially along the body in the ectoderm, and circumferentially in the endodermic layer. Contraction of ectodermal myoepithelial cells results in a decrease in length of the body column and tentacles. On the contrary, contraction of the myoepithelial cells in the endoderm produces the lengthening of these structures [18,36,47]. These contractile cells

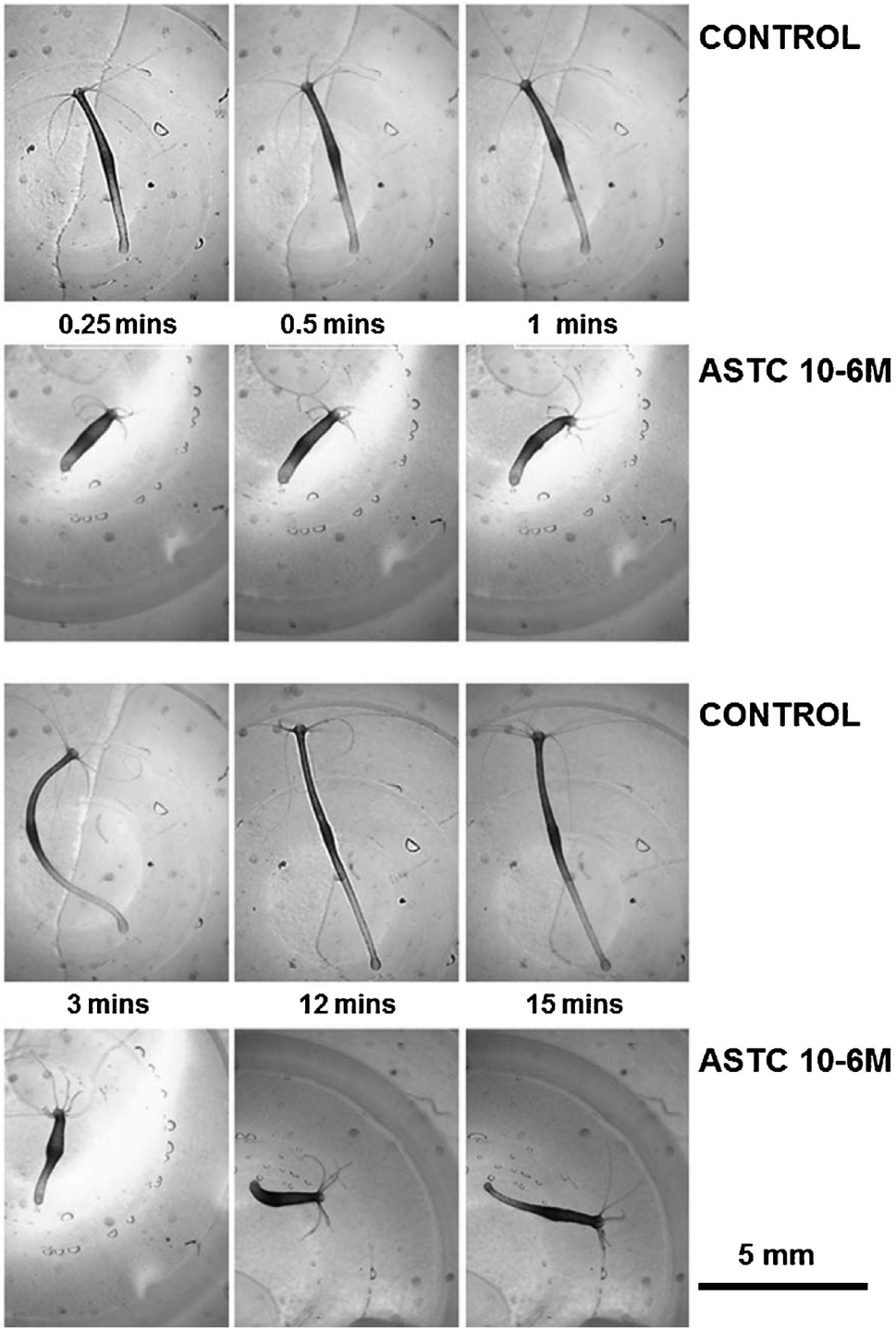

Fig. 2. Overall response of *Hydra* sp. undergoing AST-C treatment. The images show selected moments along a time lapse treatment of a hydroid with AST-C 10^{-6} M compared with the control. Note the changes in the length of the tentacles, peduncle and the total body size of the treated hydroid compared with a control. Changes in the orientation of the tentacles and the shape of the GVC are also evident.

are innervated, and the synapses have elements that resemble the neuromuscular junctions of the bilaterians [9].

The peptides present in cnidarians can be classified into two groups, namely epithelial peptides and neuropeptides. The first group is involved in morphogenesis [35], while neuropeptides are mainly involved in neurotransmission; acting directly or indirectly on epithelial muscle cells to control coordinated movements. Some of the myoregulatory neuropeptides that control movement of the hydroid have been characterized. For example, *Hydra*-RFamide III acts on the contractile activity of the peduncle and regulates the movement of fluids into the gastrovascular cavity (GVC) [30]. Furthermore, neuropeptides like Hym-176, Hym-248, and FRamide 1 and 2 regulate the contraction and elongation of the body [36,37,47]. Recently, our group demonstrated the presence and myoregulatory activity of an Allatotropin-like peptide that is involved in the hipostome extrusion and feeding behavior in *Hydra* sp. [3]. Allatotropin (AT) is a pleiotropic neuropeptide, stimulating juvenile hormone (JH) synthesis [17], as well as visceral muscle

contractions and dorsal vessel frequency in several insect species, including *Rhodnius prolixus* and *Triatoma infestans* [28,33,43].

The presence of AT-like peptides in both Cnidaria and Platyhelminthes, strongly suggests that the ancestral function of this peptide is related with muscle activity regulation, being the regulation of JH synthesis a synapomorphic feature of insects [2,3,12]. Allatostatins (ASTs) constitute a group of neuropeptides that has been originally characterized because of their inhibitory effect on the synthesis of JHs in insects. A member of this group of neuropeptides, Allatostatin-C (AST-C), originally characterized in *Manduca sexta* (Lepidoptera, Insecta), displays myoregulatory functions in several insect species (for a review see Ref. [6,42]).

In the present study we analyzed the effect of *Aedes aegypti* AST-C in *Hydra* sp., a freshwater member of the phylum Cnidaria. AST-C conjugated with Qdots were utilized to reveal target tissues and specific cell populations that respond to the peptide in different regions of hydroids, suggesting the presence of specific receptors for this peptide in *Hydra* sp. Furthermore, physiological assays using different concentration of AST-C, showed that the peptide induces changes in shape and length of different body regions of the hydroids (*i.e.* tentacles, body and peduncle, as well as the GVC). The observed changes were dose and time dependent, suggesting the physiological nature of the responses. Altogether, these results suggest that AST-C has a myoregulatory role in *Hydra* sp., being involved in the control of coordinated movements. Therefore, both AT and AST-C would be ancestral peptides playing roles in myoregulatory control.

2. Materials and methods

2.1. Animals

Individuals of *Hydra* sp. were obtained from a colony maintained in dechlorinated water at 20 ± 2 °C with a 12:12 h light/dark period. Animals were fed with *Artemia salina*. For physiological assays, specimens were starved during the 72 h previous to the experiment. All the experiments were performed with groups comprised by 7 individuals. Each specimen was kept isolated throughout the entire experiment.

2.2. Quantum dot peptide conjugation

Quantum dot nanocrystals (Qdot) conjugated to streptavidin (1 μM) were purchased from Quantum Dot Corporation (Hayward, CA). Their peak of emission is at 605 nm (red). Biotinylated *Aedes aegypti*-AT and AST-C were custom synthesized (Biopeptide, San Diego, CA). Qdot nanocrystals were conjugated to 5–10 streptavidin molecules, with a total of 20–40 binding sites for biotin. Each biotinylated neuropeptide stock was dissolved at 0.92 mM in 100% DMSO (Dimethylsulfoxide, Sigma) and stored at −70 °C.

An excess of neuropeptides (4.3 μl) was added to 10 μl of QDs, along with 58.7 μl of incubation buffer (2% BSA in 50 mM borate buffer pH 8.3 and 0.05% NaN3). The mixture was incubated for 1.5 h at room temperature. To remove the excess of free peptide, Qdot-peptide conjugates were washed five times with 0.5 ml of phosphate buffered saline (PBS) in a Microcon-100 concentrator (Amicon Bioseparations, Bedford, MA, USA) at 12,500*g* 5 min. The Qdot-peptide conjugates were recovered in 100 μl of PBS and stored at 4 °C.

2.3. In vivo *binding assays using Qdot conjugates*

As previously reported, groups of hydroids were incubated in a solution containing either Qdot-AT or Qdot-AST-C conjugates, or in a solution containing non-conjugated Qdot nanocrystals (control group) [3,38] for 30 min, and then fixed in formaldehyde-PBS (4%) at 4 °C for 12 h [3]. Samples were analyzed at 543 nm with a Laser Scan Confocal Microscope (LSCM) Zeiss LSM 510 Meta.

2.4. Physiological assays

Hydroids were treated with different doses of *A. aegypti* AST-C (QIRYRQCYFNPISCF) [21]. Groups of seven hydroids were starved for 72 h, then individually placed in fresh water and acclimated for 10–15 min. Once the experimental specimens were acclimated, the solution was replaced with water containing AST-C at different concentrations (10^{-14}, 10^{-12}, 10^{-10} 10^{-8}, 10^{-6} and 10^{-5} M). The same individual was used as a control placing it in fresh water before adding different doses of peptide solutions. Each dose was tested for 15 min using the same hydroid. Before testing the next concentration, hydroids were allowed to return to control conditions.

The experimental specimens were examined individually under a binocular microscope, and their activities were recorded with digital video camera. A time-lapse was recorded for each experiment by taking a picture every 3 s during 15 min. The body, peduncle, GVC and tentacles length of individual hydroids were measured at different time points during the 15 min of exposition to each dose (*i.e.* 0.15, 0.30, 1, 3, 5, 8, 10, 12 and 15 min) by using the GNU Image Manipulation Program (GIMP) software [19]. For the GVC evaluation, the maximum width/length ratio was measured and the presence/absence of contractions was also determined. The length of tentacles was measured as the distance comprised between their distal end and the mouth.

2.5. Statistics

Differences between treatments were analyzed by multifactorial ANOVA. Taking into account that the number of tentacles of the different hydroids varies, for the analysis of the length tentacle, each tentacle of each hydroid was individually measured, being also the hydroid considered as a factor. In this way, the differential response for every dose along the time could be evaluated. Single post-hoc comparisons were tested by the least significant difference (LSD) method. For the analysis of the percentage of contractions, Chi-square test was used. Only differences equal or less than 0.05 were considered significant.

2.6. Identification of putative Allatostatin-C receptor orthologues

A protein BLAST search in Cnidaria and different phyla of Metazoa was performed in GenBank using the complete sequence of *M. sexta* and *A. aegypti* AST-C-receptor (AST-Cr), and the signature sequence of somatostatin/AST-C receptors located in the TM7 domain [23,27]. Only those sequences that presented the complete transmembrane domains and the characteristic **E/DR** motif associated to the TM3 were included. Finally, four sequences pertaining to phylum Cnidaria (*i.e. Nematostella vectensis*, *Acropora digitifera* and *Hydra vulgaris*) were selected. Sequences representative of the main groups of Metazoa that fitted the above detailed features were also included (*i.e.* Arthropoda; Priapulida; Mollusca; Annelida; Echinodermata; Chordata) (Supplementary File 1 in the online version at DOI: 10.1016/j.peptides.2016.05.011).

The selected sequences were aligned using the Clustal Wallis algorithm (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and further analyzed by the software JalView 2.7 [45]. The analysis of the probable evolutionary relationships between sequences was performed by the use of Mega 6.06 software [34] based on the complete transmembrane domains sequences (amino and carboxyl terminals were excluded) [4,8], using the Maximum likelihood method based on the Poisson correction model, including a 1000 replicates bootstrap analysis.

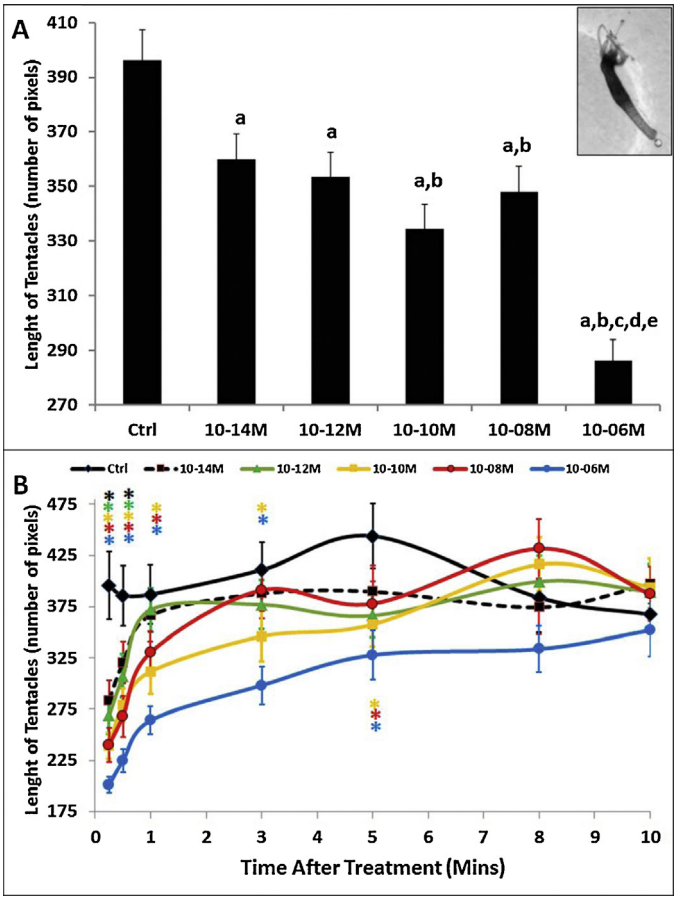

Fig. 3. Effect of AST-C treatment on the tentacles of *Hydra* sp. (A) Average change of the length of the tentacles induced by different doses of AST-C in starved hydroids along a 10 min period. (B) Changes in the length of the tentacles induced by each dose applied at different moments along the experiment. Note that high doses elicited a stronger reaction for a longer period. Inset. General aspect of a hydroid treated with AST-C 10^{-5} M. Note that the tips of the tentacles are oriented to the mouth. Each point represents mean ± SE (n = 7). Colored asterisks represent significant differences with the length of the control for each dose applied at the corresponding time registered.

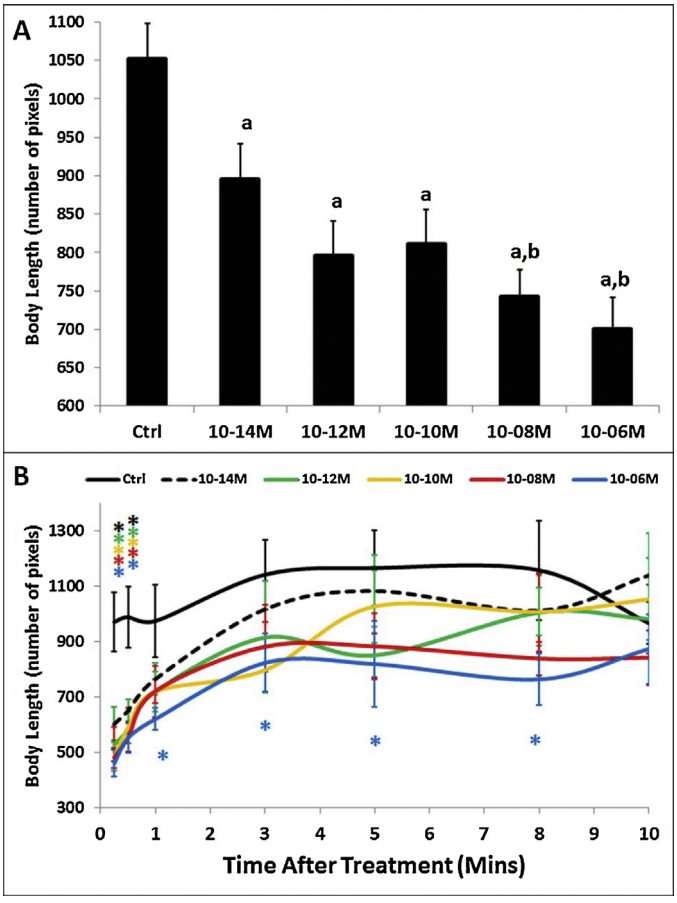

Fig. 4. Effect of AST-C treatment on the length of the body. (A) Average changes of the total body length induced by different doses of AST-C. (B) Shortening of the total body length induced by each AST-C dose measured at different moments along the experiment. As in Fig. 3, higher doses cause a stronger shortening and a longer effect. Each point represents mean ± SE (n = 7). Asterisks represent significant differences with the length of the control for each dose applied at the corresponding time registered.

3. Results

3.1. AT and AST-C Qdot labeling

Hydroids incubated in a solution containing unconjugated Qdots did not generate any signal (data not shown), showing that the binding to cells was specific for the Qdot-peptide conjugates.

Microscopic analysis of the tentacles showed that AT and AST-C conjugates were specifically associated to cells presenting different distribution in the tissues (Fig. 1A and B). Notably, the distribution of AST-C labelled cell populations at the tentacles was clearly different from that observed for AT conjugates. In orthogonal cuts of the tentacles, the distribution of both peptides reveals that AST-C signal is present in cell populations located in the inner layer of the tentacle (Fig. 1C), while the observed distribution for the AT conjugates, was in the outer layer (Fig. 1D). Furthermore, the distribution of AT and AST-C labeled cells is different, showing a circular pattern for the AT recognizing cells (Fig. 1B), demonstrating the specificity of the reactions of both peptides. Finally, in a magnified view, not only cells distribution but also size and aspect of the cells that recognize each peptide seems to be different (Fig. 1E and F).

3.2. Effect of AST-C on the tentacles motility

The histological analysis suggested the existence of cell populations that respond specifically to AST-C, therefore we decided to analyze the dose and time-dependent effects of AST-C on tentacle movements on groups of hydroids starved during 72 h. (Figs. 2 and 3).

AST-C treatment induced changes in the length of tentacles (measured as the distance from their distal end to the mouth), as well as in their shape; resembling the changes produced by the presence of food. On the contrary, tentacles in control hydroids remained relaxed.

Low doses (10^{-14} and 10^{-12} M) shortened the length of the tentacles only during the first 30 s, returning to the relaxed condition 1 min after treatment (Fig. 3B). Doses ranging between 10^{-10} and 10^{-6} M extended the effect during 3–5 min (Fig. 3B). A high dose (10^{-5} M) shortened tentacle length with the tip oriented to the mouth during the entire experiment (inset in Fig. 3A).

3.3. Effect of AST-C on the length of the body and peduncle

The same individuals were used to analyze the dose and time-dependent effect of AST-C on the length of the body and peduncle. AST-C also produced significant changes on the total length of the body and the peduncle in a dose and time-manner (Figs. 2, 4 and 5).

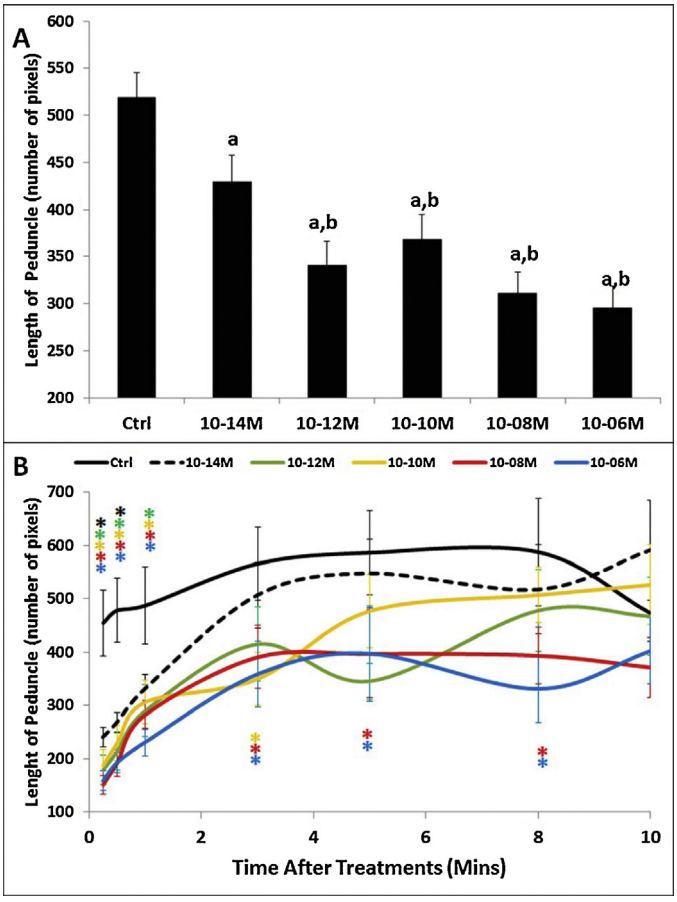

Fig. 5. Effect of AST-C treatment on the peduncle of *Hydra* sp. (A) Dose-response average changes of the length of the peduncle induced by AST-C. (B) Decrease of the length induced by each AST-C dose applied at different moments along the experiment. Each point represents mean ± SE (n = 7). Asterisks represent significant differences with the length of the control for each dose applied at the corresponding time registered.

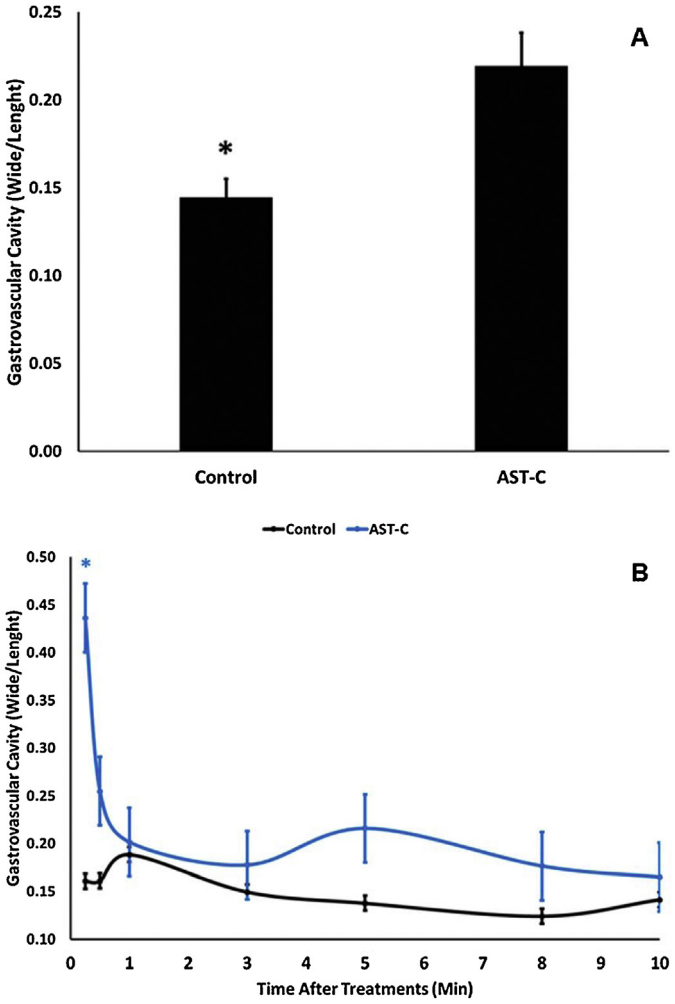

Fig. 6. AST-C induced changes on the shape of the gastrovascular cavity. (A) Overall change induced by AST-C 10^{-6} M measured during a 10 min period (mean ± SE). The changes in shape were evaluated as the maximum wide/length ratio. (B) Time dependence of the effect, showing a strong, but not sustained in time, response of the cavity. Each point represents mean ± SE (n = 7) of the W/L ratio. Asterisk represents statistically significant differences between hydroids undergoing AST-C treatment and controls 15 s after the treatment was applied.

Doses ranging between 10^{-14} to 10^{-8} M induced a significant decrease of body length, sustained only during the first 30 s after treatment. However, the effect remained up to 8 min at 10^{-6} M (Fig. 4B).

AST-C also induced a statistically significant dose-dependent decrease in the length of the peduncle with all the doses assayed (Fig. 5A). The effect was also dependent on the elapsed time, being the reduction of the peduncle length maintained for longer times as the dose of the peptide was increased (Fig. 5B).

3.4. *Effect of AST-C on the gastrovascular cavity peristaltic-like contractions and shape*

AST-C did not induce any significant change on the length, or the maximum wide of the GVC. However, at a concentration of 10^{-6} M of AST-C, a significant change on the shape of the GVC was evidenced when the maximum wide/length ratio was evaluated (Fig. 6A). As it is shown in Fig. 6B this change was sustained only for a few seconds, being statistically significant only for the first 15 s after treatment.

As we described above, the highest concentration assayed (10^{-5} M AST-C) further increased the effect of the peptide on all the variables described above (*i.e.* tentacles, body and peduncle) reaching a full and sustained contraction that was persistent throughout the period of 15 min analyzed. On the contrary, at the GVC this dose induces recurrent contractions on most of the hydroids that underwent the treatment (Fig. 7A).

A detailed analysis of the time-lapse at this concentration showed that, in 5 out of seven hydroids the treatment triggered contractions that resembled peristaltic waves (Fig. 7B–E). As shown in the movie (Supplementary File 2 in the online version at DOI: 10.1016/j.peptides.2016.05.011) the peristaltic-like waves observed, began at a given point below the hypostome, moving down along the GVC in a direction orientated to the proximal end of the hydroid. Indeed, this kind of movements was reiterated several times throughout the period assayed.

3.5. In silico *search for Allatostatin-C receptors in Cnidaria and their probable phylogenetic relationships with groups of Bilateria*

We identified four sequences pertaining to three different species of Cnidaria (*N. vectensis* and *A. digitifera* (Anthozoa) and *H. vulgaris* (Hydrozoa)) that shared significant similarity with the AST-C receptor of *A. aegypti* and other insect species. The sequences show a high degree of identity at the proposed signature of the AST-C/somatostatin-like receptor family contained in TM7 (Table 1). The alignment of these sequences, together with

Table 1
Aedes aegypti Allatostatin-C receptors and putative orthologues identified in Cnidaria. The table includes the percentage of identity and similarity of the sequence considered as a signature for the AST-C/Somatostatin receptor family and accession numbers. Red letters show amino acids residue identity. Blue letters show conservative and semiconservative changes.

Name	Class	Phylum	Signature Sequence	% identity	% similarity	Accession
A. aegypti	Insecta	Arthropoda	YSNSAMNPILYA	--------	----------	XP_001662510
N. vectensis	Anthozoa	Cnidaria	YSNSAMNPYLYA	91.7	91.7	XP_001639218
A. digitifera	Anthozoa	Cnidaria	YSNSAMNPVLYG	83.3	91.7	XP_015775303
H. vulgaris	Hydrozoa	Cnidaria	YANSALNPILYV	75.0	91.7	XP_012553580
H. vulgaris	Hydrozoa	Cnidaria	YINSALNPILYV	75.0	83.3	XP_012553585

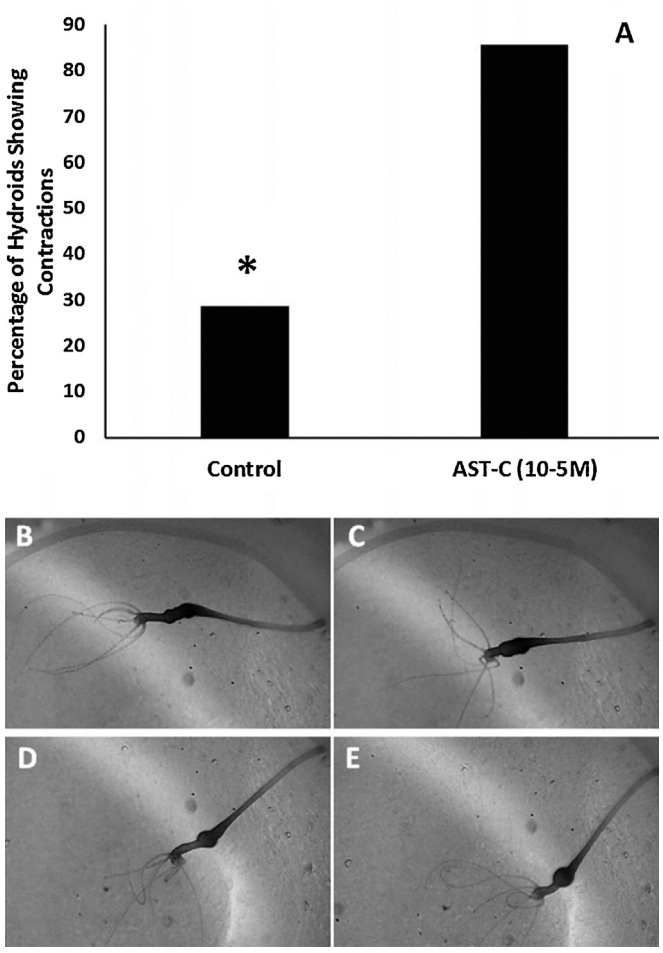

Fig. 7. Contractions of the gastrovascular cavity induced by a high dose of the AST-C peptide. (A) AST-C applied at the highest dose assayed (10^{-5} M) induces statistically significant differences in the rate of contractions of the GVC walls when compared with controls. Differences were tested by non-parametric Chi-squared test. (B–D) Images selected at different moments along the treatment, showing the sequential changes in the shape of the cavity (see also movie in Supplementary File 2).

others pertaining to several species of protostomia and deuterostomia phyla is shown in Supplementary File 3. All the sequences analyzed include the **E/DR** motif associated to TM3 that characterized GPCRs (Supplementary File 3 in the online version at DOI: 10.1016/j.peptides.2016.05.011). A phylogenetic analysis of the total of metazoan sequences showed that the three cnidarian species cluster together. Indeed, the rest of the species of Bilateria, appear clearly grouped in two clusters. These two clusters representing deuterostomia and protostomia groups share a common ancestor (Fig. 8).

Inside the bilaterian branches, *S. purpuratus* is grouped with the species of the phylum Chordata. On the other hand, species pertaining to the two major groups of protostomia resulted well clustered, representing the accepted phylogenetic relationships (*i.e.* Lophotrocozoa and Ecdysozoa). Inside the group conformed by Lophotrocozoa species, the only sequence of Mollusca that we could use in the analysis because the coincidence with the pre-stablished criteria, was grouped with three sequences of Annelida including *C. teleta* and *P. dumerilii*. Another two sequences of *P. dumerilii*, share a common ancestor conforming a separated cluster. These two sequences presented several changes (see alignment in Supplementary File 3). Finally, the analysis showed the Ecdysozoa phyla as the sister group of Lophotrocozoa (Fig. 8).

4. Discussion

Neurosecretory cells exerting paracrine or yuxtracrine regulation appeared early in evolution, preceding the origin of neuronal and endocrine systems [2,32]. It has been proposed that neuropeptides showing both excitatory and inhibitory effects are the most ancient myoregulatory messengers, being present in Cnidaria, [15,24,35–37,47]. Peptidic messengers, such as RFamide and insulin-like peptides are also present in Placozoa [26,29].

Biological molecules conjugated with nanocrystals have been previously used in *Hydra* sp. to demonstrate both, the existence of GSH binding proteins in the endodermal cell layer of the gastric region [38], and the existence of myoepithelial cell populations involved in the motility of the hypostome [3]. In fact, a previous study revealed that an AT-like peptide acts as a myoregulator in *Hydra*, exerting its effect on epithelial muscle cells, being involved in feeding behavior [3]. Indeed, as it was previously shown in other region of the hydroids [3], the present studies revealed that *A. aegypti* AST-C and AT are recognized by cells that show different distribution in the tentacle, showing the specificity of the physiological responses observed and suggesting the existence of an AST-C-like peptide in Cnidaria.

The addition of the different concentrations of AST-C generated a significant change in tentacle length and shape, with the tip of the tentacles moving up toward the mouth, which resembles the position adopted during the capture of the prey. Interestingly, with the highest dose assayed (10^{-5} M), the tentacles underwent a full and sustained contraction for a period longer than the total period recorded.

Our analysis of peduncle and GVC movements clearly showed that length changes were mainly produced by the shortening of the peduncle. It has been proposed that the movement of fluids into and out of the GVC is not produced by diffusion, but by the

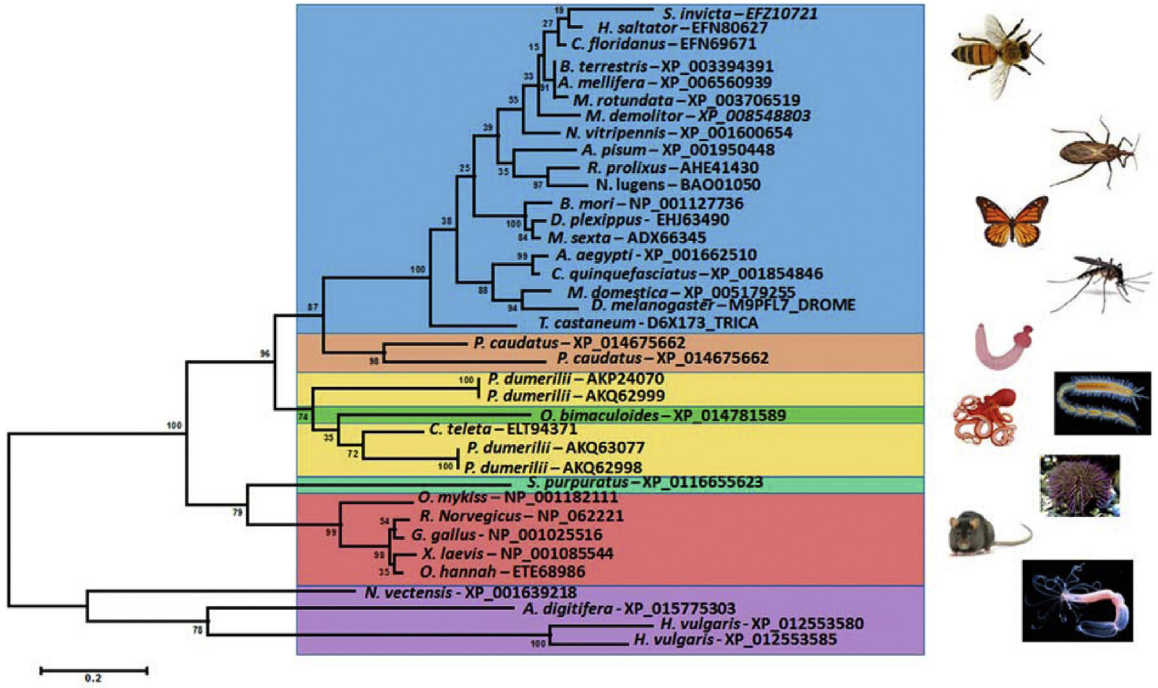

Fig. 8. Phylogenetic analysis of Somatostatin/AST-C receptors and probable orthologues in Cnidaria and other phyla. The phylogram shows the relationships between different species of the more representative phyla of Metazoa including Arthropoda, Chordata, Annelida, Priapula, Mollusca, Cnidaria and Echinodermata. The analysis is based on the region of the sequence comprised between transmembrane domains 1–7. No amino and carboxyl regions were included. The analysis was performed by Maximum likelihood methodology based on the Poisson correction model. The tree represents the consensus after 1000 replicates bootstrap. Numbers on the branches indicate bootstrap percentage after 1000 replications in constructing the tree. The genetic distances are referred to the scale. Each color in the graph identifies different phyla.

peduncle generating alternative contraction and relaxation, which produces a pumping-like effect. In this way, the peduncle could be working like a heart, being responsible of the delivery of nutrients throughout the body [30].

When AST-C 10^{-6} M was applied, the GVC changed to a bottle-like shape, which resembles the appearance adopted by the cavity after ingestion of the prey, suggesting a role of AST-C in the feeding process.

Overall, as the increment in the intensity and duration of the effect associated with the dose applied is considered characteristic of the receptor-mediated response, the dose and time-dependent effect observed suggests the physiological nature of the process.

The highest dose assayed induced a set of contractions that resembled the peristaltic waves that occur in the digestive system of most organisms (see Supplementary File 2 in the online version at DOI: 10.1016/j.peptides.2016.05.011). The existence of complex and coordinated peristaltic movements in *Hydra* sp. during digestion was previously reported [31]. The activity of neuropeptides acting as myoregulators during the digestive process is widely distributed in all phyla. In fact, it has been recently suggested that the neuropeptide MIP activates ingestion and gut peristalsis in Annelida [46].

In spite that it is known that AST-C is predicted in genomes of invertebrates other than insects [39,40], the expression and their biological activities have not been still analyzed, being in our knowledge this study the first one showing physiological activities of AST-C in other animal group beyond Insecta.

The bioinformatic search for similar sequences to the AST-C peptide or its pro-peptide in cnidarians did not show any relevant and significant sequence to be considered as a putative orthologue of this neuropeptide. Indeed, homology-based searches are often not sensitive enough to detect precursors of small neuropeptides [26]. In fact, due to these difficulties, analyses based in the functional and evolutionary relationships between different peptides have been previously described based on the orthology of their receptors. For example, an orthologous relationship between allatostatin-A and galanin is supported by the orthology of their receptors and similar roles [7,16,20]. Indeed, the existence of GPCRs in cnidarians and even in the placozoan *T. adhaerens*, has been previously shown [3]. Now, *in silico* search for probable AST-C receptors in cnidarians showed that three genomes of Hydrozoa and Anthozoa species, predict the existence of proteins showing GPCRs characteristic features, sharing a high percentage of identity at the level of the sequence considered as a signature of the AST-C/Somatostatin receptors [23,27]. Phylogenetic analysis showed that the sequences predicted by the genomes of Anthozoa are nearer to Bilateria than the sequences pertaining to *H. vulgaris* (Hydrozoa), and in fact, it has been proposed that Anthozoa is the group of cnidarians more closely related to bilaterians [10,22]. As it was mentioned above, two sequences predicted by the genome of *P. dumerilii* clustered separately, conforming the sister group of the other sequences corresponding to Lophotrocozoa. As these sequences share several distinctive substitutions, they might be representing isoforms.

It is accepted that in insects, holometabolous species are monophyletic, whilst the hemimetaolous condition would appeared several times during evolution [5]. Interestingly, as it was previously shown, hymenopteran species did not cluster with the rest of holometabolous species, appearing as a sister group of Hemiptera (hemimetabolous) [44].

Altogether, our result strongly suggest that an AST-C-like based mechanism, could be involved in the regulation of different kind of movements associated with feeding behavior in *Hydra*, providing more evidence about the ancestral functions of the neuropeptides.

Contributors

Conceived and designed the experiments: JRR MEA. Performed the experiments: MEA JRR. Analyzed the data: MEA JRR SHM. Con-

tributed reagents/materials/analysis tools: JRR SHM. Wrote the paper: JRR MEA. Critically revised the manuscript: JRR MEA SHM.

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