The Journal of Experimental Biology 207, 1803-1810
Published by The Company of Biologists 2004
doi:10.1242/jeb.00974

Control of planula migration by LWamide and RFamide neuropeptides in *Hydractinia echinata*

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Accepted 10 March 2004

Summary

Planula larvae of *Hydractinia echinata* (Cnidaria) settled on a substratum migrate toward light. We observed that planula migration is not a continuous process. Instead, it consists of repeating cycles of active migration (about 8 min on average) and inactive resting periods (about 26 min on average). This pattern of periodic migration is regulated by LWamide and RFamide neuropeptides. LWamide (10^{-8} mol l^{-1}) stimulates migration primarily by making the active periods longer, whereas RFamide (10^{-7} mol l^{-1}) inhibits migration by blocking the initiation and also shortening the length of the active periods. Since sensory neurons containing LWamides and RFamides are present in planula larvae, it appears likely that planula migration is regulated by the release of endogenous neuropeptides in response to environmental cues.

Key words: *Hydractinia echinata*, planula migration, RFamide neuropeptide, LWamide neuropeptide.

Introduction

Hydractinia echinata is a colonial marine hydroid closely related to freshwater hydra. Fertilized eggs of this species undergo rapid cleavage divisions for about a day and develop into spindle-shaped planula larvae in about 3 days (Plickert et al., 1988). The planula larvae metamorphose into adult polyps when they receive appropriate environmental stimuli (Müller, 1973; Leitz, 1998a,b).

Planula larvae have a simple neuronal network consisting of about 450 neurons (Plickert et al., 1988). About one-tenth (or less) of them contain neuropeptides of the LWamide family, which share the common C-terminal sequence Gly-Leu-Trp-NH_2 (Leitz et al., 1994; Schmich et al., 1998). A similar, or somewhat smaller, number of neurons contain neuropeptides of the RFamide family sharing the C-terminal sequence Gly-Arg-Phe-NH_2 (Grimmelikhuijzen, 1985; Plickert, 1989). Neurons containing LWamides or RFamides are located in the ectodermal cell layer. Their cell bodies are oval and slender in shape and the tip of one end of the long cell bodies appears to be exposed to the external environment, suggesting that majority (if not all) of these cells probably function as sensory cells to monitor the environment. The cell bodies are localized exclusively in the anterior half, near the blunt-end of the planula body, and extend long processes into the posterior half. These processes are relatively thin, run mainly parallel to each other, have few varicosities, and do not form extensive cross-links (Plickert, 1989; Leitz and Lay, 1995; Schmich et al., 1998).

Previous studies have shown that LWamides induce metamorphosis of planula larvae to adult polyps (Leitz et al., 1994; Gajewski et al., 1996; Takahashi et al., 1997). We have recently shown that RFamides inhibit metamorphosis induced by LWamides or other inducing agents (Katsukura, 1998; Katsukura et al., 2003). Thus, LWamides and RFamides work antagonistically to each other in regulating metamorphosis in planula larvae of *H. echinata*.

LWamides and RFamides have also been shown to act as neurotransmitters (or neuromodulators) in sea anemone. Both neuropeptides induce rhythmic contraction in small pieces of parietal muscle tissue isolated from sea anemone (McFarlane et al., 1987, 1991; Takahashi et al., 1997). Whether or not LWamides and RFamides similarly stimulate muscle contraction in planula larvae of *H. echinata* is presently not known. This led us to examine the possible functions of these peptides as neurotransmitters (or neuromodulators) in planulae.

The muscle-stimulating activity of LWamides and

Fig. 1. Planula migration. Images of a single planula larva migrating toward light were captured at the rate of 1 image per 30 s and superimposed to create one figure. Note that the specimen oriented the blunt end of its spindle-shaped body toward light and that contraction and relaxation of tissue along the body axis accompanied migration. The specimen shown was migrating faster (1.2 mm min^{-1}) than average (0.3 mm min^{-1}, see Fig. 5C) thus facilitating visualization of changes in shape and position during migration. Scale bar, 1 mm.

RFamides in sea anemone was examined using isolated muscle tissue. Similar experiments with planula larvae are not feasible since they are too small (about 1 mm in length) to isolate muscle tissue. Therefore we used planula migration (Müller, 1969) as an alternative assay for muscle activity. The planula of *Hydractinia* is covered by ciliated epitheliomuscular cells (Weis et al., 1985). We presume that ciliary activity is the basis for planula migration. In addition, it will be shown that contraction and relaxation of epitheliomuscular cells probably also play an important role in migration (see Fig. 1).

We placed planula larvae in a glass container and allowed them to migrate towards light on the bottom surface of the container. We then captured images of migrating planulae at regular intervals and analyzed the migration process by analysis of the images.

Our results show that planula larvae do not migrate continuously. Instead, they undergo alternating phases of active migration and inactive resting behavior. LWamide stimulates migration primarily by increasing the length of the active periods and secondarily by increasing the speed of migration during the active periods. In contrast, RFamide inhibits migration by reducing the initiation and the length of active periods. Both neuropeptides produce these effects at concentrations that are one to two orders of magnitude lower than those affecting metamorphosis (Katsukura et al., 2003).

Possible mechanisms for regulating both migration and metamorphosis by the LWamides and RFamides are discussed.

Materials and methods

Animals

The strains of *Hydractinia echinata* used and their culture were described by Katsukura et al. (2003). Briefly, a pair of male (M4) and female (F5) strains of *H. echinata* growing on flat scallop shells (5–7 cm in diameter) were maintained in a tank containing about 300 liters of natural seawater. Fertilized eggs obtained from this culture were allowed to develop to planula larvae at 18°C in flat-bottomed glass bowls placed on a reciprocal shaker set at 70 strokes min^{-1} with a 2.5 cm path. All experiments were carried out using 4- or 5-day old planula larvae obtained in this way.

Image analysis

Migratory movement of planula larvae was examined using an image recording system consisting of a dissecting microscope (Nikon SMZ-U, Tokyo, Japan), a 3-CCD video-camera (Hitachi HV-C20S, Tokyo, Japan), an analog/digital converter (Sony DVMC-MS1, Tokyo, Japan) and a computer (Macintosh G4). A small water bath maintained at 18°C was placed directly under a dissecting microscope. A glass spectrophotometer cell having 30 mm light path and 10 mm width was used as a container for observations of planula migration. The planula larvae were placed in 3 ml of seawater in the cell and kept undisturbed overnight at 18°C. Next day, the cell was gently moved to the water bath (18°C) under the dissecting microscope, and light was introduced from a metal halide lamp through a glass fiber cable into the cell from one side. Light intensity was approximately 3000 lx at the entrance into the cell. Planula larvae, initially randomly located on the bottom surface of the spectrophotometer cell were attracted toward the light source at one end of the cell after approximately 2 h. At this time, the orientation of the spectrophotometer cell was gently reversed, placing the majority of animals near the end of the spectrophotometer cell away from the light source.

A small volume (30 µl) of seawater containing (or not containing) a peptide (see below) was then gently added and images of planula larvae migrating towards the light along the 30 mm light path were captured at the rate of 1 frame per 30 s during a 2 h period (in 240 consecutive frames). NIH *Image* (version 1.62) was then used to analyze planula migration. Planula larvae have a slender spindle-shaped body with a broad blunt shape at the anterior end and a slender tapered tip at the posterior end (see Fig. 1). We used the blunt end to define the position of animals in migration experiments. The location of the blunt end in the *X–Y* coordinates of the NIH *Image* was determined for each step of migration (from frame 1 to 240) for each animal. A set of data obtained in this way for each animal was then transferred to Microsoft Excel (version 98) and used to carry out the following analyses.

Migration track (see Fig. 2) was prepared by connecting the animal's positions in the *X–Y* coordinate from frame 1 to frame 240 (or the last frame when the animal reached the end of light path).

Actogram of migration (see Fig. 3) was prepared by plotting individual 'step lengths' as a function of observation time. The step length (mm), defined as the distance migrated between two consecutive frames, was calculated from Equation 1,

where x_n and y_n represent the animal's position in the *X*–*Y* coordinate in frame *n*:

$$\text{Step length} = [(x_n - x_{n-1})^2 + (y_n - y_{n-1})^2]^{1/2} . \quad (1)$$

Track length (mm), defined as the sum of the individual step lengths over a period of 1 h, was calculated by Equation 2:

$$\text{Track length} = \sum [(x_n - x_{n-1})^2 + (y_n - y_{n-1})^2]^{1/2} . \quad (2)$$

Length of active periods (min) was calculated as the average length (min) of all individual active periods during the period of observation.

Number of active periods was calculated as the average number of individual active periods per hour.

Speed of migration during active periods (mm min^{-1}) was calculated by dividing the sum of individual step lengths in an active period by the length of the active period.

Peptides

Hydra-RFamide I (pGlu-Trp-Leu-Gly-Gly-Arg-Phe-NH_2; Moosler et al., 1996) and He-LWamide II (Lys-Pro-Pro-Gly-Leu-Trp-NH_2; Gajewski et al., 1996) were used as representative members of the RFamide and LWamide families, respectively, as described by Katsukura et al. (2003).

Results

Planula migration

Planula larvae of *H. echinata* migrate toward light (Müller, 1969). A typical example of migration is presented in Fig. 1. It shows a series of 16 consecutive images of a single migrating planula larva captured at the rate of 1 image per 30 s. The 16 images were then combined into one image to show the changes in shape and position of the specimen during migration. The migrating animal oriented its blunt end toward light and moved its slender body like a whip, suggesting that the planula propelled itself by ciliary activity as well as by coordinated contraction and relaxation of the tissue along its body axis.

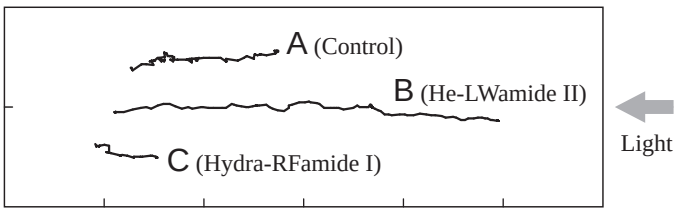

Fig. 2. Migration tracks of planula larvae in (A) normal seawater, (B) seawater containing 10^{-8} mol l^{-1} He-LWamide II, or (C) seawater containing 10^{-7} mol l^{-1} Hydra-RFamide I. The three tracks shown were obtained from independent experiments, but are shown together to aid comparison. The rectangular box surrounding the tracks represents the spectrophotometer cell with 30 mm light path and 10 mm width that was used as a container to observe planula migration.

Track and actogram of migration

A typical example of the migration track (Materials and methods) made by a planula in normal seawater in 2 h is presented in Fig. 2A. The track is 11.9 mm in length and nearly straight from a starting point on the left to an end point on the right. Fig. 3A is an actogram (Materials and methods) for the same specimen showing the step lengths (distance moved between 2 consecutive frames) as a function of time for 120 min. The striking feature of the actogram is that planula moved periodically with active periods of migration (5–10 min) separated by resting periods (10–20 min). Similar features, i.e. movement in a nearly straight line toward the light source and repeating cycles of active and inactive migration, were observed in almost all the specimens examined (see below).

Effects of He-LWamide II and Hydra-RFamide I on the migration of planula larvae

He-LWamide II, a member of the LWamide neuropeptide family (Materials and methods), stimulated migration at very low concentrations. Fig. 2B shows the track made by a specimen in seawater containing 10^{-8} mol l^{-1} He-LWamide II during a 120 min period. The track appears similar to that made by the control planula in normal seawater (Fig. 2A), but its length (20.4 mm) is significantly longer. The actogram for the same specimen (Fig. 3B) indicates that the active periods (13–15 min) are considerably longer than those of the control animal (6–15 min) (Fig. 3A).

Hydra-RFamide I, a member of RFamide neuropeptide family (Materials and methods), also affected migration, but in the opposite way from He-LWamide II. The track (4.0 mm,

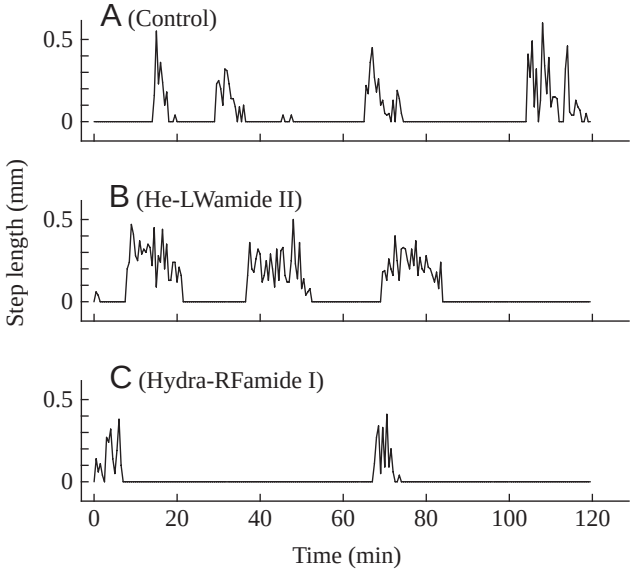

Fig. 3. Actogram of migration for planula larva in (A) normal seawater, (B) seawater containing 10^{-8} mol l^{-1} He-LWamide II, or (C) seawater containing 10^{-7} mol l^{-1} Hydra-RFamide I. The time of observation is plotted against step length (distance moved between two frames: 30 s).

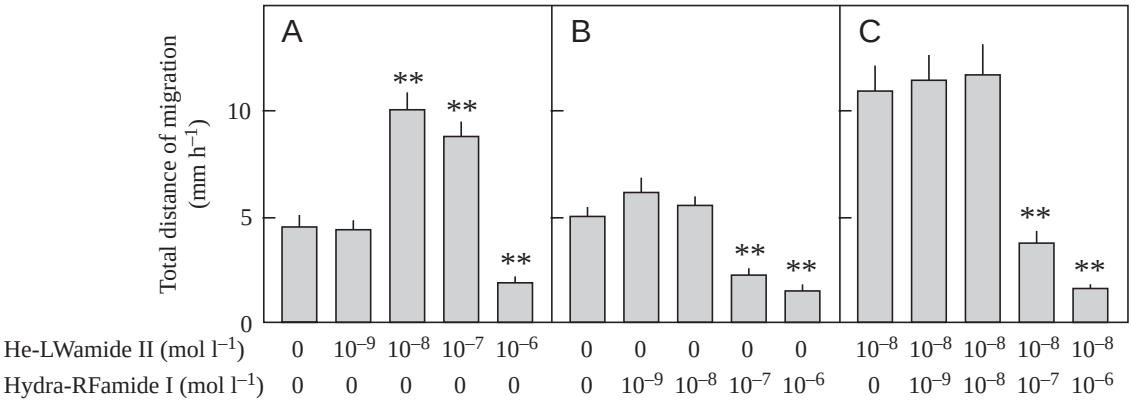

Fig. 4. Effect of He-LWamide II and Hydra-RFamide I on track length. Peptide concentration is plotted against the distance migrated during 1 h (track length). Values shown are means + S.E.M. Asterisks indicate results that are significantly different from the control values (shown in the far-left column in each panel) (*t*-test; **P*<0.05, **0.01). Sample size was minimally 50.

Fig. 2C) made by a planula in seawater containing 10^{-7} mol l^{-1} Hydra-RFamide I was significantly shorter than that of the control animal. The active periods were also generally shorter (7 min; Fig. 3C) and fewer in number than in control animals.

Planulae also migrated in the dark. This could be observed by placing a single planula in a container, leaving the container in the dark for a fixed period of time (e.g. 60 min) and comparing the locations of the specimen at the start and end of the period. Planula migration in the dark was random in direction, i.e. planulae moved in all possible directions from the starting site. The migration track, however, could not be followed by the image analysis procedure employed in this study. For this reason the effect of the LWamide or RFamide on migration in darkness was not investigated.

Quantitative analysis of planula migration

The results shown in Figs 2 and 3 demonstrate the basic effects of He-LWamide II and Hydra-RFamide I treatment on the migration of representative planula larvae. To obtain more quantitative data we examined larger numbers of specimens at different concentrations of the two peptides. The results are presented in Figs 4 and 5.

Fig. 4A shows the effect of He-LWamide II on the track length. The average length of tracks made by control planula larvae in normal seawater was 4.6 mm. This length was roughly doubled to 10.2 and 8.8 mm, respectively, by treatment with 10^{-8} and 10^{-7} mol l^{-1} He-LWamide II. Treatment with 10^{-6} mol l^{-1} He-LWamide II significantly shortened the track length. Since He-LWamide II induces metamorphosis at this concentration (see fig. 2A in Katsukura et al., 2003), this strong negative effect on migration was probably produced secondarily by the induction of metamorphosis (see Discussion).

By comparison, Hydra-RFamide I treatment significantly

Table 1. *Summary of the effects of He-LWamide II and Hydra-RFamide I on migration of* H. echinata

		[He-LWamide II] (mol l^{-1})			[Hydra-RFamide I] (mol l^{-1})	
Parameters of migration	Dimension	0 (Control)	10^{-8}	10^{-7}	0 (Control)	10^{-7}
Track length (observed)	mm h^{-1}	4.6 (1)	10.2 (2.2)	8.8 (1.9)	5.1 (1)	2.3 (0.5)
Number of active periods (*N*)	h^{-1}	1.8 (1)	1.8 (1)	1.7 (0.9)	1.7 (1)	1.1 (0.6)
Length of active periods (*L*)	min	8.0 (1)	15.3 (1.9)	14.5 (1.8)	8.7 (1)	6.6 (0.8)
Speed of migration during active periods (*S*)	mm min^{-1}	0.32 (1)	0.41 (1.3)	0.38 (1.2)	0.33 (1)	0.31 (0.9)
Track length (calculated as *N*×*L*×*S*)	mm h^{-1}	4.6 (1)	11.3 (2.5)	9.4 (2.0)	4.9 (1)	2.3 (0.5)

The numbers in parentheses show ratios relative to the control values at 0 mol l^{-1} set at 1.

reduced track length (Fig. 4B). The average track length for control animals in this experiment (5.1 mm) was reduced to 2.3 mm at a concentration of 10^{-7} mol l^{-1} Hydra-RFamide I. This length was further reduced to 1.5 mm at 10^{-6} mol l^{-1}, but this is again the concentration affecting metamorphosis (fig. 2B in Katsukura et al., 2003).

To look for possible interactions between He-LWamide II and Hydra-RFamide I, we treated planulae with both peptides simultaneously, using concentrations that do not affect metamorphosis. As shown in Fig. 4C, the increased track length produced by He-LWamide II treatment at 10^{-8} mol l^{-1} (11.0 mm) was significantly reduced by simultaneous treatment with Hydra-RFamide I at 10^{-7} mol l^{-1} (3.8 mm), suggesting that He-LWamide II and Hydra-RFamide I work antagonistically to each other.

Track length is controlled by three separate parameters: the number of active periods, the length of active periods, and the speed of migration during active periods (Materials and methods). The effects of both peptides on each of these three parameters were analyzed using concentrations that do not affect metamorphosis. The results are presented in Fig. 5, and summarized in Table 1.

Fig. 5A shows the effect of He-LWamide II on the number of active periods per hour. The number, which was about 1.8 for control animals in normal seawater, was unaffected by He-LWamide II at any concentration used. In contrast, the length of individual active periods (Fig. 5B) was significantly increased from roughly 8.0 min in control animals to 15.3 and 14.5 min in animals treated with 10^{-8} and 10^{-7} mol l^{-1} He-LWamide II, respectively. The average speed of migration during active periods was also increased, but only moderately, from 0.32 mm min^{-1} in control animals in normal seawater to 0.41 and 0.38 mm min^{-1} in animals treated with 10^{-8} and 10^{-7} mol l^{-1} He-LWamide II, respectively (Fig. 5C).

Fig. 5D–F shows the results of a similar analysis for Hydra-RFamide I. This peptide significantly reduced the track length

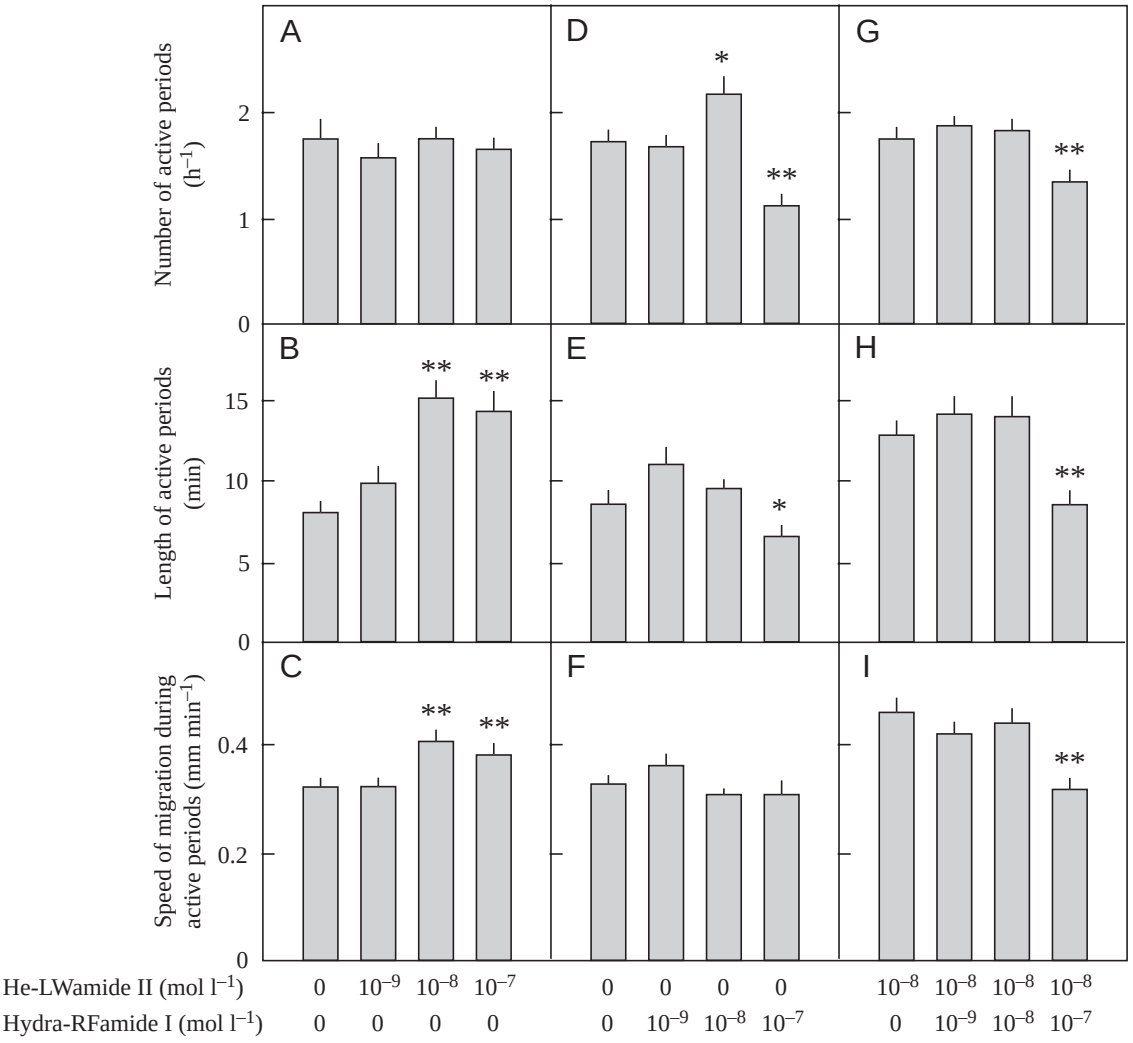

Fig. 5. Effect of various concentrations of He-LWamide II and Hydra-RFamide I on three parameters of migration: the number of active periods per hour (A,D,G), the length of active periods (B,E,H), and the speed of migration during active periods (C,F,I). Asterisks indicate results that are significantly different from the control values (shown in the far-left column in each panel) (*t*-test; **P*<0.05, **0.01). Sample size was minimally 50.

at 10^{-7} mol l^{-1} (Fig. 2C). At the same concentration, it also reduced the number of active periods from the control value of 1.7 to 1.1 per hour (Fig. 5D) and the length of individual active periods from the control value of 8.7 min to 6.6 min (Fig. 5E). However, it had no effect on the speed of migration (Fig. 5F).

Simultaneous treatment with He-LWamide II (Fig. 5G–I) and Hydra-RFamide I produced basically similar effects to those observed with single Hydra-RFamide I treatment (Fig. 5D–F), except that Hydra-RFamide I treatment alone had little effect on the speed of migration (Fig. 5F) whereas simultaneous treatment with He-LWamide II caused a moderate reduction in the speed (Fig. 5I).

Discussion

Planula larvae of *Hydractinia* scan the environment for suitable settlement sites by migrating over the substratum. They have two types of neurons, one containing LWamides and the other containing RFamides. Both types appear to be sensory neurons, based on morphology. They are localized in the anterior end of planula body (Plickert, 1989; Leitz, 1993; Leitz and Lay, 1995; Gajewski et al., 1996; Schmich et al., 1998). The presence of these sensory-type neurons suggests that the neuropeptides they contain might affect the migration behavior of planulae. Our results confirm this hypothesis. Planula larvae move in a periodic manner: active phases of migration are interspersed with resting phases during which no movement occurs. LWamides at 10^{-8} mol l^{-1} stimulate migration primarily by increasing the duration of the active phases. LWamides also produce moderate positive effects on the speed of migration, but no effects on the number of active phases. In contrast, RFamides at 10^{-7} mol l^{-1} decrease the initiation and duration of active phases and thus inhibit migration (Figs 4 and 5). Thus, LWamides and RFamides act antagonistically to each other to regulate migration.

Uptake of LWamides and RFamides by planula tissue

Ectodermal epithelial cells of hydra polyps are connected to each other by septate junctions, providing a permeability barrier to the polyp tissue (Wood, 1979). A similar permeability barrier presumably also exists in *Hydractinia* planulae. Nevertheless, peptides isolated from various cnidarian sources exert their effects when externally applied to intact polyps or planulae (see, for example, Schaller and Bodenmueller, 1981; Leitz et al., 1994; Takahashi et al., 1997; Bosch and Fujisawa, 2001; Katsukura et al., 2003). How these peptides penetrate, or bypass, the permeability barrier and exert their effect on intact animal is not presently understood.

The dose response of LWamides and RFamides has been previously examined using small pieces of muscle tissue isolated from sea anemone. LWamides and RFamides stimulated muscle contraction weakly at 10^{-8} mol l^{-1} and strongly at 10^{-7}–10^{-6} mol l^{-1} (McFarlane et al., 1987, 1991; Takahashi et al., 1997). By comparison, LWamide strongly stimulated planula migration at 10^{-8} mol l^{-1} (Fig. 4A) and RFamide strongly inhibited migration at 10^{-7} mol l^{-1} (Fig. 4B). Thus, LWamides and RFamides were active at similar concentrations both in the *in vitro* muscle stimulating system, which lacks a permeability barrier, and in the intact planula migration system. These observations suggest that the planula epithelium does not represent a significant permeability barrier. Thus, planulae, and also hydra polyps, have some mechanism that allows uptake of peptides from the external medium.

LWamide and RFamide neuropeptides play different roles depending on concentration

Katsukura (1998) and Katsukura et al. (2003) have shown that, at higher concentrations than used here, the LWamides and RFamides also act antagonistically to regulate metamorphosis in *H. echinata*. LWamides at 10^{-6} mol l^{-1} induce while RFamides at 10^{-5} mol l^{-1} inhibit metamorphosis. Taken together, these and our present observations suggest that LWamide and RFamide neuropeptides are bifunctional peptides that play two different roles in the same organism. At relatively low concentrations they affect migration, while at higher concentrations they control metamorphosis.

A similar situation has been described previously for cAMP in the slime mold *Dictyostelium*. In this organism, cAMP at 10^{-8}–10^{-9} mol l^{-1} acts as a chemoattractant to stimulate aggregation of single amoebae to form large aggregates. In these developing aggregates high concentrations of cAMP (10^{-7} mol l^{-1}) control cell differentiation. These two effects are mediated by different cAMP receptors having different affinities for cAMP and different cytoplasmic domains (Parent and Devreotes, 1996).

Neuropeptides having more than one function have been described in vertebrates. For example, the hypothalamic PACAP peptide, which stimulates adenylate cyclase activity in the pituitary gland of mammals, promotes proliferation of mouse primordial germ cells in culture (Pesce et al., 1996). Substance P, which is involved in pain sensation in the central and peripheral nervous system, stimulates the proliferation of rabbit intervertebral disc cells in culture (Ashton and Eisenstein, 1996). ANP peptides, which regulate Na^+ homeostasis in adult tissue, accelerate proliferation of chick myocardial cells in culture (Koide et al., 1996).

Two different functions have been known for the LWamides for some time. LWamides induce metamorphosis in *Hydractinia* (Leitz et al., 1994; Gajewski et al., 1996; Takahashi et al., 1997) and also stimulate muscle contraction in the sea anemone *Anthopleura* and in *Hydra* (Takahashi et al., 1997). Based on these observations, Takahashi et al. (1997) suggested that LWamides play two different roles depending on developmental stages in cnidarians, serving as morphogenetic factors to regulate metamorphosis in embryonic stage and as neurotransmitters (or neuromodulators) to stimulate muscle contraction in polyp stage.

Our results now show that LWamides and RFamides can play two different roles depending not on different developmental stages, but on different concentrations. They

regulate planula migration at relatively low concentrations and metamorphosis at higher concentrations.

How is this achieved? A complete molecular explanation is not possible at present, since we do not know which cells are targets for the LWamide and RFamide effects nor do we know if the target cells for metamorphosis and migration are the same or different. At least, however, the different concentration dependence of the metamorphosis and migration effects appears to require the presence of receptors with different affinities for both peptide families in a similar manner to the cAMP receptors in *Dictyostelium* described above. And the receptors with different affinities are presumably connected to different signal transduction cascades and could be in different cell types.

Alteromonas *bacteria may be the natural signal controlling both metamorphosis and planula migration*

The fact that LWamides and RFamides play two different roles in planula larvae suggests that planula larvae may use the same environmental cues to control both migration and metamorphosis. Marine bacteria of the genus *Alteromonas* present on hermit crab shells are thought to serve as the environmental cue triggering metamorphosis (Müller, 1973; Leitz and Wagner, 1993; Plickert et al., 2003). Some other marine bacteria also have the capacity to trigger metamorphosis (Kroiher and Berking, 1999). We have recently obtained preliminary results showing that *Alteromonas* bacteria can stimulate planula migration. Interestingly, this effect occurs at a tenfold lower bacterial concentration than that required for metamorphosis induction (data not shown). Thus, *Alteromonas* bacteria may serve as the cue for both migration and metamorphosis, depending on bacterial density in the natural environment. Similarly, RFamide-containing neurons may respond to an environmental cue, leading to release of RFamides into tissue and inhibition of migration or metamorphosis. However, the identity of this putative factor is presently unknown.

Control of migration

Our results have uncovered a unique feature of periodicity in planula migration. Active phases of migration are invariably interspersed with resting phases. What is the mechanism that generates this periodicity in migration? We have no answer to this question at present, but can consider a few possible mechanisms. For example, the periodicity in migration simply could be due to periodic changes in the physiological state of migrating planulae, e.g. periodic depletion of components (for example AMP or calcium ion) necessary for migration activity.

Periodicity could be also be generated by more sophisticated mechanisms such as interaction between LWamide- and RFamide-containing neurons. For example, the LWamide-containing neurons could be excitatory neurons, which release LWamide to stimulate contractile tissue. In addition, the same LWamides might also stimulate the inhibitory RFamide-containing neurons. The RFamides released could then act back on the LWamide-containing neurons to downregulate their activity. If this type of cross-catalytic interaction exists between the excitatory and inhibitory neurons, it could generate a periodicity of migration in planula larvae. However, no evidence presently exists for direct interaction between the LWamide- and RFamide-containing neurons.

New assay systems to examine neuropeptide levels in tissue, their release from neurons in response to stimuli from within or without, and the response of various cell types in the tissue to the neuropeptides, will provide a better understanding of the regulatory mechanisms controlling the complex behavior of migration and metamorphosis.

T.S. was a recipient of Alexander von Humboldt-Foundation Research Award, which enabled him to initiate this project in the laboratory of C.N.D. This work was supported in part by grants from Sumitomo Foundation and Yamada Science Foundation to T.S. and by grants from the German Science Foundation (DFG) to C.N.D.

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