Helgol Mar Res (2000) 54:230–236
DOI 10.1007/s101520000053

ORIGINAL ARTICLE

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Induction of metamorphosis from the larval to the polyp stage is similar in Hydrozoa and a subgroup of Scyphozoa (Cnidaria, Semaeostomeae)

Received: 12 June 2000 / Accepted: 4 September 2000 / Published online: 16 November 2000

Abstract Larvae of cnidarians need an external cue for metamorphosis to start. The larvae of various hydrozoa, in particular of *Hydractinia echinata*, respond to Cs^+, Li^+, NH_4^+ and seawater in which the concentration of Mg^{2+} ions is reduced. They further respond to the phorbolester, tetradecanoyl-phorbol-13-acetate (TPA) and the diacylglycerol (DAG) diC8, which both are argued to stimulate a protein kinase C. The only well-studied scyphozoa, *Cassiopea* spp., respond differently, i.e. to TPA and diC8 only. We found that larvae of the scyphozoa *Aurelia aurita*, *Chrysaora hysoscella* and *Cyanea lamarckii* respond to all the compounds mentioned. Trigonelline (*N*-methylnicotinic acid), a metamorphosis inhibitor found in *Hydractinia* larvae, is assumed to act by delivering a methyl group for transmethylation processes antagonising metamorphosis induction in *Chrysaora hysoscella* and *Cyanea lamarckii*. The three species tested are scyphozoa belonging to the subgroup of semaeostomeae, while *Cassiopea* spp. belong to the rhizostomeae. The results obtained may contribute to the discussion concerning the evolution of cnidarians and may help to clarify whether the way metamorphosis can be induced in rhizostomeae as a whole is different from that in hydrozoa and those scyphozoa belonging to the subgroup semaeostomeae.

Keywords Cnidaria · Metamorphosis · *Aurelia aurita* · *Cynanea lamarckii* · *Chrysaora hysoscella*

Introduction

Many marine invertebrates have a larval stage and have to undergo metamorphosis from the larval to the adult stage. Generally, an external cue is necessary for metamorphosis to start, in particular for those animals whose larvae are free-floating while the adults are fixed to a surface or are slow moving. In most cases a biofilm makes a substrate suitable for metamorphosis induction (for review see Fletcher 1994). In marine environments almost all substrates are covered by biofilms. Within the biofilm certain bacteria are suggested to deliver the metamorphosis-inducing stimulus (for review see Chia and Bickell 1978; Clare et al. 1998). In some species there is a high substrate specificity, e.g. in *Cassiopea xamachana* (scyphozoa) (Fleck and Fitt 1999); but in others there is not. In the hydrozoon *Hydractinia echinata* various Gram-positive and Gram-negative bacteria isolated from marine environments including the common *Escherichia coli* have been shown to induce metamorphosis effectively (Kroiher and Berking 1999).

In hydrozoa, metamorphosis is induced by several agents, including Li^+ and Cs^+ ions, which are neither present in sufficient concentrations in the larva nor in its natural habitat. Nevertheless, these agents are thought to be excellent tools to obtain access to the biochemical pathways linked to metamorphosis control. At best that strategy will result in the detection of all pathways which somehow affect the decision to metamorphose. With respect to *H. echinata*, the most studied member of the hydrozoa, up to now about 400 substances have been applied to larvae under controlled conditions (by various authors with quite different experimental intentions). Only a few of them induce metamorphosis, including Li^+, K^+, Cs^+, Rb^+, Ba^{2+}, Sr^{2+}, (but not Ca^{2+}), tetraethylammonium, NH_4^+ and methylamine, vanadate, amiloride, several phorbolesters, some diacylglycerols, LWamide peptides, and compounds which antagonise polyamine synthesis. Further, Mg^{2+} deficiency and a temperature shift cause metamorphosis (for review see Berking 1998).

Only few species of scyphozoa have been studied with respect to metamorphosis induction because usually the larvae are difficult to obtain. One has to collect the rather large medusae from the open sea. Certain substrates were found to stimulate metamorphosis of *Aurelia*

Communicated by H.-D. Franke

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aurita (Korn 1966; Brewer 1978; Keen 1987). A more detailed analysis was performed with *Cassiopea* spp. The larvae of these animals can be induced to metamorphose by treatment with the phorbolester, tetradecanoylphorbol-13-acetate (TPA) (Fleck and Bischoff 1992) and two artificial diacylglycerols, diC6 and diC8 (D.K. Hofmann, personal communication). The inorganic ions found to cause metamorphosis in *H. echinata* have been tested and have failed to cause metamorphosis in *Cassiopea andromeda* larvae (Fitt et al. 1987). The larvae respond to specific peptides (Fitt and Hofmann 1985; Fleck 1997) but not to those that *H. echinata* (Leitz and Lay 1995) responds to (D.K. Hofmann, personal communication). Thus, scyphozoa and hydrozoa seem to use somewhat different pathways for metamorphosis induction.

In this study we used the scyphozoa *Aurelia aurita*, *Chrysaora hysoscella* and *Cyanea lamarckii*. We tested different substances which initiate metamorphosis in the hydrozoa *H. echinata*. We selected them to test the involvement of a broad spectrum of possible biochemical pathways. Two of the substances (TPA and diC8) are thought to act by direct interference with protein phosphorylation. One (Li^+) is postulated to interfere with the phosphatidylinositol pathway. Two other inorganic ions (NH_4^+ and Cs^+) are assumed to act by increasing, directly or indirectly, the internal concentration of ammonium ions. Seawater with a reduced content of Mg^{2+} ions was also tested. A convincing hypothesis concerning which pathway is primarily affected by this treatment is still needed (for review see Berking 1998). We further tested the effect of trigonelline, which is suggested to stabilise the larval state as an endogenous compound in the hydrozoon *H. echinata* (Berking 1986).

Materials and methods

The experiments were performed at the Biologische Anstalt Helgoland (BAH).

Animals

Adult medusae (jellyfish) of *A. aurita*, *Ch. hysoscella*, *C. lamarckii* and *C. capillata* were collected from the North Sea at Helgoland, and put separately into an aquarium at 18°C. The next day, all the released larvae were collected and transferred to Petri dishes with filtered (0.4 µm) seawater.

Induction of metamorphosis and treatment with test substances

For a certain period of time, generally 3 h (exceptions are mentioned in the figure legends), the larvae (about 35 larvae in 3 ml medium) were exposed to seawater containing a certain concentration of a putative inducer dissolved in seawater. The test solutions were made iso-osmolaric to seawater and adjusted to pH 8.1. TPA and the diacylglycerol diC8 were predissolved in DMSO. (The respective controls included DMSO.) Mg^{2+}-reduced seawater was prepared according to Müller (1985). After incubation, the dishes were washed three times with fresh seawater. After 24 h the metamorphoses were scored (exceptions are mentioned in the figure legends). Metamorphosing animals stuck to the water–air interface or to the dish and underwent the transformation into a polyp, while the larvae moved as usual. For each substance concentration, the experiment was performed in triplicate. The experiment was repeated at least twice. Because of the restricted availability of competent larvae some experiments were performed with two species only.

Significance (P<5%) was tested by means of χ^2 analysis. The bars in the figures indicate the confidence interval (95%).

Results

General features of larvae, metamorphosing animals and primary polyps

The larvae of the investigated species were drop or spindle shaped. Those of *A. aurita* and *C. lamarckii* had a rather uniform length of about 300 and 200 µm, respectively. Larvae of *Ch. hysoscella* were highly variable in length. Some had a length below 100 µm while others in the same batch had a length above 500 µm. The primary polyps, too, differed in shape. Due to its long stalk, the polyp of *C. lamarckii* measured about 600 µm in length while that of *A. aurita* measured about 200 µm. The size of the primary polyps of *Ch. hysoscella* scattered around a mean of 350 µm. The very small larvae were excluded from the experiments because we suspected quantitative differences in the response of larvae of different sizes.

Some larvae underwent metamorphosis "spontaneously". The frequency was different in different species. It was comparatively high in *A. aurita*, but differed considerably between larvae obtained from different medusae. Furthermore, it was very high in the fourth species

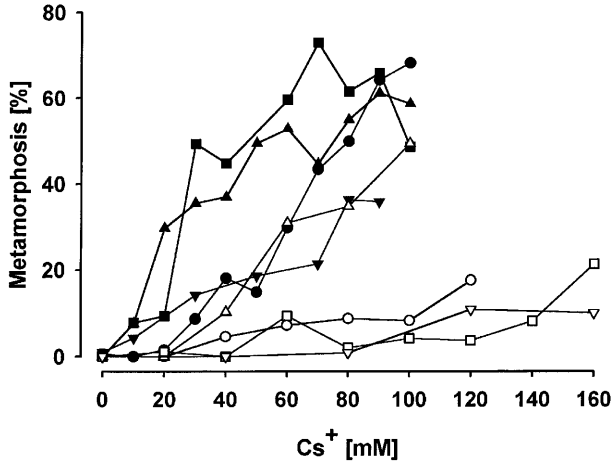

Fig. 1 Induction of metamorphosis in *Cyanea lamarckii*. Cs^+-enriched seawater was applied to the larvae for 3 h in the noted concentrations. The *ordinate* gives the resultant frequency of metamorphosing animals. Each of the *different symbols* represents larvae obtained from different medusae of *C. lamarckii* except for the *two types of closed triangles*. The latter were obtained from the same medusa but used for the experiment on different days. *Closed symbols* refer to experiments done in July 1997, *open symbols* refer to experiments done in July 1999. Each symbol represents the determinations of the developmental stages of at least 100 animals

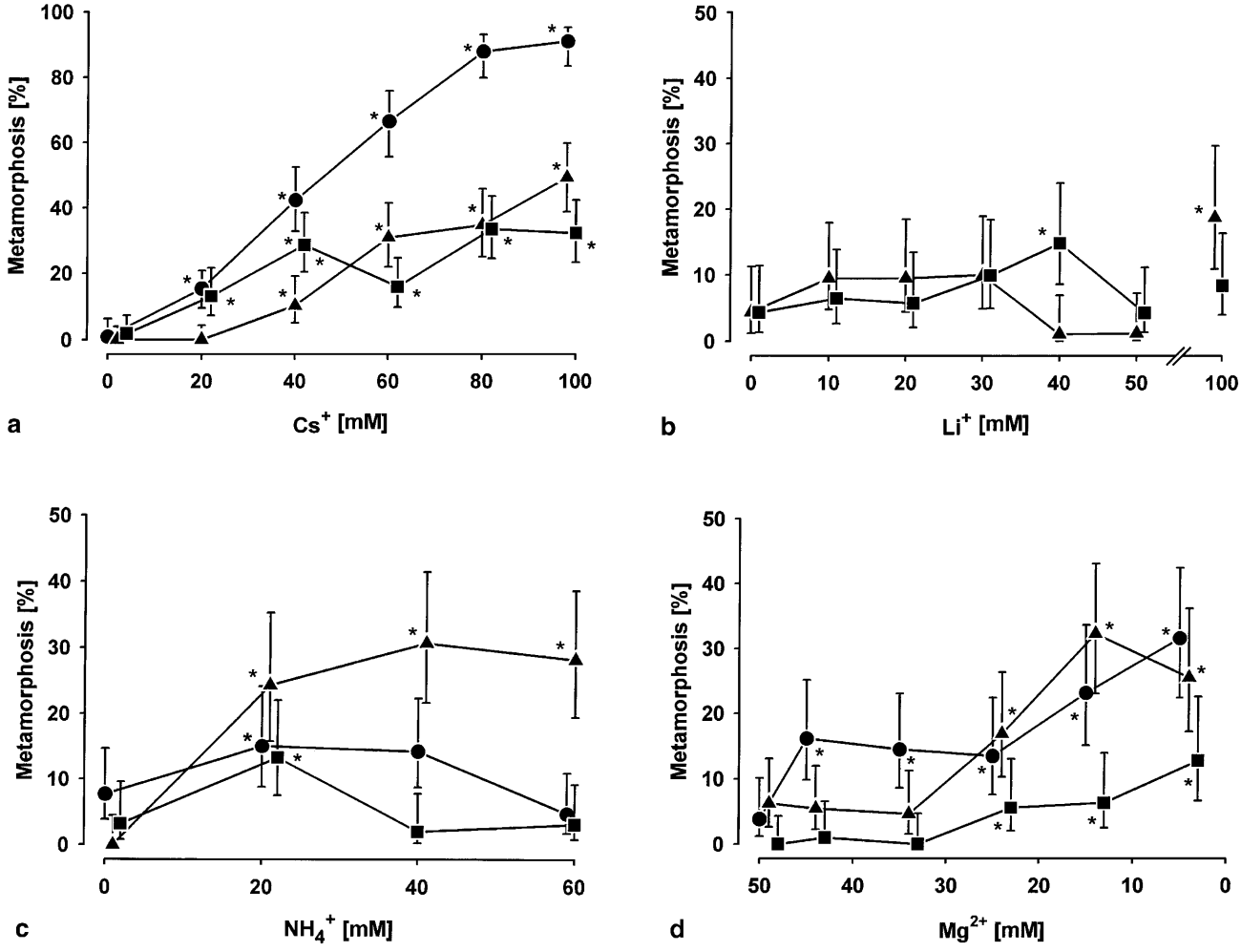

Fig. 2a–d Induction of metamorphosis in *Aurelia aurita* (●), *Chrysaora hysoscella* (■) and *Cyanea lamarckii* (▲) by inorganic ions. *Ordinate* as in Fig. 1. *Abscissa* Concentrations of the chemicals which have been applied to larvae for 3 h. ★ indicates a significant difference between the result of the treated group and that of the respective control (χ^2 analysis, $P<5\%$). The *bars* in the figures indicate the confidence interval (95%). **a** Treatment with Cs^+-enriched seawater. **b** Treatment with Li^+-enriched seawater. Scored 72 h after treatment. **c** Treatment with NH_4^+-enriched seawater. Scored 48 h after treatment. **d** Treatment with Mg^{2+}-reduced seawater. Scored 48 h after treatment. (Natural seawater contains about 50 mM Mg^{2+} ions)

investigated, *C. capillata*. Because of this, we did not use larvae of these animals in our experiments.

Following induction of metamorphosis, the resultant primary polyps were found at the bottom of the dish or hanging upside down from the water–air interface. Following treatment with Cs^+-enriched seawater, in *C. lamarckii* about 20% were found at the bottom, in *A. aurita* it was 40% and in *Ch. hysoscella* about 80%.

The speed of transformation from the larva into a polyp was different in each species. In *A. aurita* the primary polyp had developed four tentacles 1 day after metamorphosis induction. In *Ch. hysoscella* it took 2 days. In *C. lamarckii* the development lasted 3–4 days or even longer. The day following induction the metamorphosing animals did not show tentacles but looked like a disc.

The efficiency of metamorphosis induction differed in larvae obtained from various animals. Figure 1 shows the results obtained with larvae of *C. lamarckii* treated with various concentrations of Cs^+ ions in seawater. Each symbol corresponds to larvae obtained from different medusae except the two types of closed triangles. In the latter case the larvae were obtained in one batch from the same medusa but were used for the experiment on different days. Closed symbols refer to experiments done in July 1997. Open symbols refer to experiments done in July 1999, both at the same place at Helgoland. For unknown reasons the medusae collected in the summer of 1999 produced larvae which displayed a weaker response.

Induction of metamorphosis by inorganic ions

Cs^+ enrichment

Larvae of the scyphozoa *A. aurita*, *Ch. hysoscella* and *C. lamarckii* underwent metamorphosis following a 3 h treatment with Cs^+-enriched seawater in a dose-dependent manner (Fig. 2a). Larvae of *H. echinata* were found

to respond identically (Müller and Buchal 1973). The graph shows that the most sensitive species was *A. aurita* (Fig. 2a). However, the sensitivity depended considerably on the origin of the larvae (cf. Fig. 1).

Li^+ enrichment

A 3-h treatment of larvae of *Ch. hysoscella* and *C. lamarckii* with Li^+-enriched seawater caused a low but significant increase in the rate of metamorphosing animals (Fig. 2b). Concentrations above the highest one shown harmed the animals. *H. echinata* responded to Li^+-enriched seawater with metamorphosis in a dose-response curve showing an optimum at about 150 mM. Concentrations at and above the optimum harmed the animals (Spindler and Müller 1972).

NH_4^+ enrichment

A 3-h treatment of larvae of *A. aurita*, *Ch. hysoscella* and *C. lamarckii* with NH_4^+-enriched seawater caused metamorphosis (Fig. 2c). The effect was low but significant. The highest concentration shown harmed the animals. Larvae of *H. echinata* responded in a dose-response curve showing an optimum at 70 mM NH_4^+. Concentrations above the optimum, e.g. 100 mM, prevented all larvae from metamorphosing. The larvae were not harmed, rather they could be induced immediately afterwards by applying lower concentrations of NH_4^+ or other inducers to start metamorphosis (Berking 1988).

Mg^{2+} deficiency

Larvae of *A. aurita*, *Ch. hysoscella* and *C. lamarckii* underwent metamorphosis in seawater in which the concentration of Mg^{2+} ions was reduced for 3 h (Fig. 2d). The lower the concentration of the Mg^{2+} ions the higher was the frequency of inductions. *H. echinata* responds similarly (Müller 1985).

Induction of metamorphosis by agents which activate a protein kinase C

In *A. aurita*, *Ch. hysoscella* and *C. lamarckii* a 2- and a 3-h treatment with the phorbolester TPA and the diacylglycerol diC8 caused metamorphosis (Fig. 3a, b; the application of 100 µM diC8 caused some precipitation.) Similar results were obtained with *H. echinata* (Müller 1985; Leitz and Müller 1987). The three scyphozoa responded slightly more sensitively to TPA than *Hydractinia*. In *Hydractinia* 50% metamorphosis was obtained by a treatment for 2 h with 50 nM TPA.

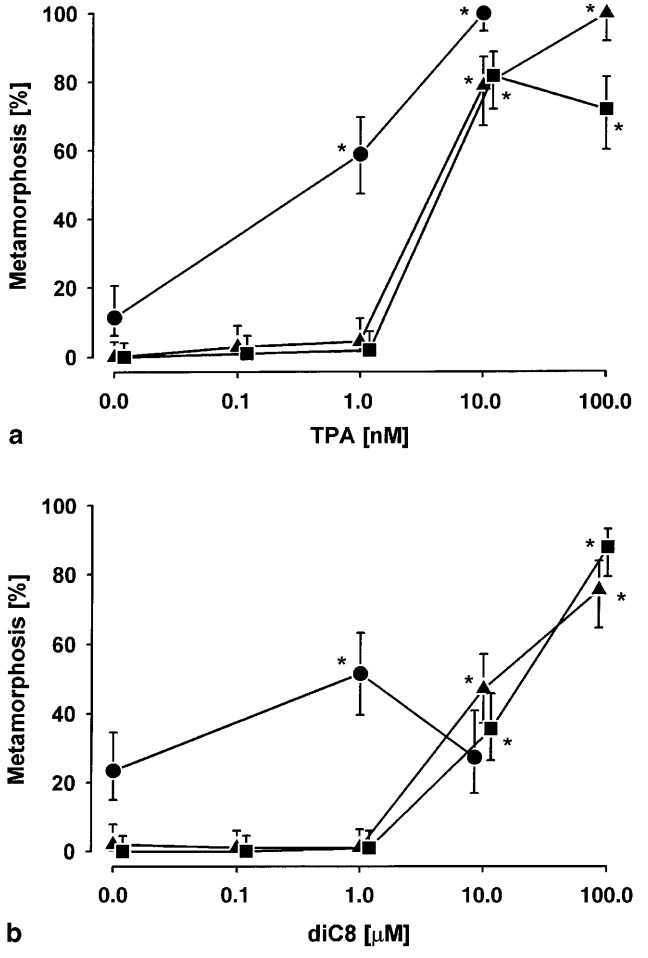

Fig. 3a,b Induction of metamorphosis in *Aurelia aurita* (●), *Chrysaora hysoscella* (■) and *Cyanea lamarckii* (▲) by compounds which stimulate a protein kinase C. Symbols and statistics as in Fig. 2. **a** Treatment with TPA-enriched seawater for 2 h. **b** Treatment with diC8-enriched seawater for 3 h

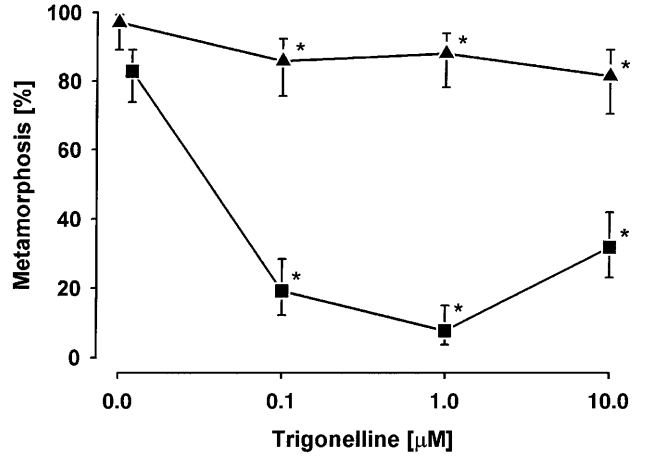

Fig. 4 In *Chrysaora hysoscella* (■) and *Cyanea lamarckii* (▲) trigonelline antagonises metamorphosis induction by Cs^+-enriched seawater. Symbols and statistics as in Fig. 2. The larvae were treated for 20 h with 12 mM Cs^+-enriched seawater and simultaneously with trigonelline at the noted concentrations. Scored 48 h after treatment

Antagonising metamorphosis induction

In the tissue of *H. echinata* substances have been found which antagonise metamorphosis induction and thereby stabilise the larval state. Some of them: homarine, trigonelline, and betaine, are thought to act by delivering a methyl group for transmethylation and/or aminopropylation processes (Berking 1986; Walther et al. 1996). We tested one of them, trigonelline, and found it to antagonise metamorphosis induction in *C. lamarckii* and *Ch. hysoscella* (Fig. 4). As in *H. echinata*, the larvae had been treated with low concentrations of Cs^+-enriched seawater (12 mM) and simultaneously with trigonelline for 20 h.

Discussion

In order to obtain larvae of *A. aurita*, *Ch. hysoscella* and *C. lamarckii*, the respective medusae were collected from the open sea. They did not undergo metamorphosis spontaneously when kept in filtered seawater, but had to be induced. The frequency of inductions was found to depend not only on the species investigated but also on the individual medusa of which the larvae had been obtained (cf. Fig. 1). Thus a positive response is a good argument for the ability of a substance to induce metamorphosis while a failure of induction is not a very strong argument for a substance to be unable to cause at least some induction.

Generally, in cnidarians, metamorphosis does not start spontaneously but requires an external cue. The differential distribution of the polyps (colonies) of the various cnidarians in nature points to different cues the species respond to and to differences with respect to their survival at the respective places. This study is not concerned with the natural cues but with substances which can replace them. In most cases, the various natural cues can be replaced by a treatment with the phorbolester TPA (Müller 1985; Freeman and Ridgway 1990; Henning et al. 1991; Fleck and Bischoff 1992; Thomas et al. 1997). The list of cnidarians which can be induced to metamorphose by that agent is now augmented by *A. aurita*, *Ch. hysoscella* and *C. lamarckii*. However, in cnidarians, TPA affects a large number of processes including, in the fresh water polyp *Hydra*, a change in the positional value, the prevention of foot formation at the bud's base, inhibition of nerve-cell formation during treatment and stimulation of nerve-cell formation after treatment (for review see Berking 1998) and in *Podocoryne carnea* the transdifferentiation of striated muscle cells (Kurz and Schmid 1991). In order to start metamorphosis, the treatment has to last for hours and often the animals do not look very healthy for some time afterwards. Further, TPA is effective at extremely different concentrations in the species tested: e.g. the three scyphozoa *A. aurita*, *Ch. hysoscella* and *C. lamarckii* and the hydrozoa *H. echinata* and *Mitrocomella polydiademata* were found to react about 1,000 times more sensitively than the scyphozoon *Cassiopea xamachana*. We suspect that an artificial strong and long activation of a protein kinase C causes a considerable alteration of the organism's regulatory state. Thus, it is difficult to say which role the activation of protein kinase C plays in the natural process of metamorphosis induction. Artificial diacylglycerols which also activate a protein kinase C have been found to cause metamorphosis in the hydrozoon *Hydractinia* and in the three mentioned scyphozoa *A. aurita*, *Ch. hysoscella* and *C. lamarckii* and recently in the scyphozoon *Cassiopea xamachana* as well (diC6 and diC8) (D.K. Hofmann, personal communication). The concentrations which have to be applied are also high and the periods of treatment are long for substances which are suspected to act as second messengers.

With respect to the inorganic ions there are qualitative differences. The three tested scyphozoa respond like *H. echinata*. *Cassiopea andromeda* did not respond at all (Fitt et al. 1987). LW-amide peptides which were shown to induce metamorphosis in *H. echinata* (Leitz and Lay 1995) failed in *Cassiopea* spp. (D.K. Hofmann, personal communication). Hydrozoa were found to respond very similarly but not identically: *Hydractinia symbiolongicarpus* failed to respond to ammonia (Thomas et al. 1997). The hydrozoon *Halocordyle disticha* can be induced simply by the application of permeabilising agents such as saponin and dimethylsulfoxide (Thomas et al. 1997). Several putative neurotransmitters such as DOPA, dopamine and norepinephrine, induce metamorphosis in *Halocordyle disticha*, but not in the hydrozoa *H. echinata, Mitrocomella polydiademata* and *Phialidium gregarium* (Freeman and Ridgway 1990; Thomas et al. 1997). In summary, with respect to metamorphosis induction, the three scyphozoa tested display a strong similarity to *H. echinata* and various other hydrozoa and a very low similarity to *Cassiopea* spp. Interestingly, *A. aurita*, *Ch. hysoscella* and *C. lamarckii* belong to the subgroup semaeostomeae, while *Cassiopea* spp. belong to the rhizostomeae.

How the mentioned inorganic agents and the LW-amides act is still unclear. Several of the inorganic ions (Li^+, K^+, Cs^+, Rb^+, NH_4^+) were argued to cause membrane depolarisation (e.g. Yool et al. 1986; Freeman 1993) and an increase in the internal concentration of NH_4^+ ions by antagonising the export of the produced ammonia (Berking 1988). The metamorphosis-inducing influence of ammonia is widespread among marine invertebrate larvae. Larvae of echinoids (Gilmour 1991) and of a tunicate (Berking and Herrmann 1990) were induced to metamorphose by NH_4^+ ions. In the scyphozoa *Cassiopea andromeda* it induces partial metamorphosis of a bud into a polyp (Berking and Schüle 1987). In larvae of molluscs (oyster, Japanese scallop) it induces settlement behaviour and metamorphosis (Coon et al. 1990; Kingzett et al. 1990). Ammonia is produced in the biofilm. The concentration is particularly high in grooves and pits in the substrate due to a reduced mixing of the seawater at these places (Bruland 1983). This may explain the observed preference of many marine invertebrate larvae to settle in these habitats.

In most sedentary marine species with motile larvae, either the water–air interface or a particular biofilm is argued to deliver the natural cue for metamorphosis to start. The sequence of events following that contact is yet unclear. The various artificial inducers were thus used to trace the pathways relevant in metamorphosis control. In *H. echinata*, ouabain, which blocks the Na^+/K^+-pump, was found to antagonise metamorphosis induction by Cs^+, Rb^+, NH_4^+, Li^+, vanadate and seawater in which the Mg^{2+} concentration was reduced (Müller and Buchal 1973; Müller 1985; Berking 1988; Leitz and Wirth 1991). Furthermore, ouabain antagonises the inducing influence of the bacterial film (Müller 1973). In contrast, it stimulates induction by diC8 (Berking and Walther 1994). It thus appears unlikely that bacteria or the respective bacterial products directly stimulate PKC activity.

In *H. echinata* trigonelline (*N*-methyl nicotinic acid) and two related substances, homarine (*N*-methylpicolinic acid) and betaine (*N*-trimethylglycine), were found to be stored in the tissue and to antagonise metamorphosis induction when applied externally along with the inducer Cs^+ (for review see Berking 1998). The substances were argued to act by their ability to deliver a methyl group for transmethylation or for aminopropylation of various substances. The latter can result in the polyamines spermidine and spermine, which were shown to antagonise metamorphosis in *Hydractinia* (Walther et al. 1996). In *Cassiopea andromeda* trigonelline antagonised the inducing influence of TPA and of the phosphatase inhibitor cantharidin which both can cause the transformation of a bud into a polyp, a process similar to metamorphosis (Kehls et al. 1999). Cantharidin failed to induce metamorphosis in *H. echinata*, *A. aurita*, *Ch. hysoscella* and *C. lamarckii* (unpublished results). Because the larvae of the three tested semeaostomeae respond to trigonelline it is argued that they make use of the same pathways in metamorphosis control as *H. echinata* does.

The question now is whether *Cassiopea* spp. are an exception or rhizostomeae as a whole respond differently. If the latter is true the differential response may contribute to the discussion concerning the evolution of cnidarians.

Acknowledgements We thank the staff at the Biologische Anstalt Helgoland for support. The experiments comply with the current laws of Germany.

References

Berking S (1986) Transmethylation and control of pattern formation in hydrozoa. Differentiation 32:10–16

Berking S (1988) Ammonia, tetraetylammonium, barium and amiloride induce metamorphosis in the marine hydroid *Hydractinia*. Roux's Arch Dev Biol 197:1–9

Berking S (1998) Hydrozoa metamorphosis and pattern formation. Curr Top Dev Biol 38:81–131

Berking S, Herrmann K (1990) Dicapryloylglycerol and ammonium ions induce metamorphosis of ascidian larvae. Roux's Arch Dev Biol 198:430–432

Berking S, Schüle T (1987) Ammonia induces metamorphosis of the oral half of buds into polyp heads in the scyphozoan *Cassiopea*. Roux's Arch Dev Biol 196:388–390

Berking S, Walther M (1994) Control of metamorphosis in the hydroid *Hydractinia*. In: Davey KG, Peter RE, Tobe SS (eds) Perspectives in comparative endocrinology. National Research Council of Canada, Ottawa, pp 381–388

Brewer RH (1978) Larval settlement behavior in the jellyfish *Aurelia aurita* (Linnaeus) (Scyphozoa: Semaeostomae). Estuaries 1:120–122

Bruland KW (1983) Trace elements in seawater. In: Riley JP, Skirrow G (eds) Chemical oceanography. Academic Press, New York, pp 157–220

Chia FS, Bickell LR (1978) Mechanisms of larval settlement and the induction of settlement and metamorphosis: a review. In: Chia FS, Rice MS (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier, New York, pp 1–12

Clare AS, Fusetani N, Jones MB (1998) Introduction: settlement and metamorphosis of marine invertebrate larvae (International symposium on settlement and metamorphosis of marine invertebrate larvae). Biofouling 12:1–3

Coon SL, Walch M, Fitt WK, Weiner RM, Bonar DB (1990) Ammonia induces settlement behaviour in oyster larvae. Biol Bull 179:297–303

Fitt WK, Hofmann DK (1985) Chemical induction of settlement and metamorphosis of the reef-dwelling coelenterate *Cassiopea andromeda*. Proc 5th Int Coral Reef Symp, Tahiti 2:239–244

Fitt WK, Hofmann DK, Wolk M, Rahat M (1987) Requirement of exogenous inducers for metamorphosis of axenic larvae and buds of *Cassiopea andromeda* (Cnidaria: Scyphozoa). Mar Biol 94:415–422

Fleck J (1997) Phosphatidylinositol (PI) signaling and subsequent events in metamorphosis induction of cnidarian larvae. Proc 8th Int Coral Reef Symp, Panama 2:1225–1229

Fleck J, Bischoff A (1992) Protein kinase C is possibly involved in chemical induction of metamorphosis in *Cassiopea* spp. (Cnidaria: Scyphozoa). Proc 7th Int Coral Reef Symp, Guam 1:456–462

Fleck J, Fitt WK (1999) Degrading mangrove leaves of *Rhizophora mangle* Linne provide a natural cue for settlement and metamorphosis of the upside down jellyfish *Cassiopea xamachana* Bigelow. J Exp Mar Biol Ecol 234:83–94

Fletcher M (1994) Bacterial biofilms and biofouling. Curr Opin Biotech 5:302–306

Freeman G (1993) Metamorphosis in the brachiopod *Terebratalia*: evidence for a role of calcium channel function and the dissociation of shell formation from settlement. Biol Bull 184: 15–24

Freeman G, Ridgway EB (1990) Cellular and intracellular pathways mediating the metamorphic stimulus in hydrozoan planulae. Roux's Arch Dev Biol 199:63–79

Gilmour THJ (1991) Induction of metamorphosis of echinoid larvae. Am Zool 31(5):105A

Henning G, Benayahu Y, Hofmann DK (1991) Natural substances, marine bacteria, and a phorbolester induce metamorphosis in the soft coral *Heteroxenia fuscescens* (Anthozoa, Octocorallia). Verh Dtsch Zool Ges 84:486–487

Keen SL (1987) Recruitment of *Aurelia aurita* (Cnidaria; Scyphozoa) larvae is position dependent, and independent of conspecific density, within a settling surface. Mar Ecol Prog Ser 38(2):151–160

Kehls NE, Herrmann K, Berking S (1999) The protein phosphatase inhibitor cantharidin induces head and foot formation in buds of *Cassiopea andromeda* (Rhizostomeae, Scyphozoa). Int J Dev Biol 43(1):51–57

Kingzett BC, Bourne N, Leask K (1990) Induction of metamorphosis of the Japanese scallop *Patinopecten yessoenensis Jay*. J Shellfish Res 9(1):119–125

Korn H (1966) Zur ontogenetischen Differenzierung der Coelenteratengewebe (Polyp-Stadium) unter besonderer Berücksichtigung des Nervensystems. Z Morphol Ökol Tiere 57:1–118

Kroiher M, Berking S (1999) On natural metamorphosis inducers of the cnidarians *Hydractinia echinata* (Hydrozoa) and *Aurelia aurita* (Scyphozoa). Helgol Mar Res 53:118–121

Kurz E, Schmid V (1991) Effects of tumor promoters and diacylglycerol on the transdifferentiation of striated muscle cells of the medusa *Podocoryne carnea* to RF-amide positive nerve cells. In: Williams RB, Cornelius PFS, Hughes RG, Robson EA (eds) Coelenterate biology: recent research on cnidaria and ctenophora. Kluwer, Dordrecht, pp 11–17

Leitz T, Lay M (1995) Metamorphosin A is a neuropeptide. Roux's Arch Dev Biol 204:276–297

Leitz T, Müller WA (1987) Evidence for the involvement of PI-signalling and diacylglycerol second messengers in the initiation of metamorphosis in the hydroid *Hydractinia echinata* Fleming. Dev Biol 121:82–89

Leitz T, Wirth A (1991) Vanadate, known to interfere with signal transduction, induces metamorphosis in *Hydractinia* (Coelenterata; Hydrozoa) and causes profound alterations of the larval and postmetamorphic body pattern. Differentiation 47(3):119–127

Müller WA (1973) Metamorphose-Induktion bei Planulalarven. I. Der bakterielle Induktor. Roux's Arch Dev Biol 173:107–121

Müller WA (1985) Tumor-promoting phorbol esters induce metamorphosis and multiple head formation in the hydroid *Hydractinia*. Differentiation 29:216–222

Müller WA, Buchal G (1973) Metamorphose-Induktion bei Planulalarven. II. Induktion durch monovalente Kationen: Die Bedeutung des Gibbs-Donnan-Verhältnisses und der Na^+/K^+-ATPase. Roux's Arch Dev Biol 173:122–135

Spindler KD, Müller WA (1972) Induction of metamorphosis by bacteria and by a lithium-pulse in the larvae of *Hydractinia echinata* (Hydrozoa). Roux's Arch Dev Biol 169:271–280

Thomas MB, Edwards NC, Ball BE, McCauley DW (1997) Comparison of metamorphic induction in hydroids. Invertebr Biol 116:277–285

Walther M, Ulrich R, Kroiher M, Berking S (1996) Metamorphosis and pattern formation in *Hydractinia echinata*, a colonial hydroid. Int J Dev Biol 40:313–322

Yool AJ, Grau SM, Hadfield MG, Jensen RA, Markell DA, Morse DE (1986) Excess potassium induces larval metamorphosis in four marine invertebrate species. Biol Bull (Woods Hole) 170:255–266