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Induction of larval metamorphosis in the ascidian, *Halocynthia roretzi* by excess potassium ion and by reduced calcium ion

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Effects of external cation concentrations on larval metamorphosis in the ascidian, *Halocynthia roretzi* were examined. Metamorphosis (tail resorption) was induced by 20 mM and 50 mM K^+ but, interestingly, was suppressed by 30 mM K^+. Low concentrations of Ca^{2+} (1–5 mM) induced metamorphosis, while high concentrations did not, in contrast to findings in other invertebrates. Moreover, BAPTA-AM, an intracellular Ca^{2+} chelator, induced metamorphosis. This suggests that a decrease in intracellular Ca^{2+} of certain cells initiates larval metamorphosis in *H. roretzi*.

Ascidian tadpole larvae attach to substrata and metamorphose after a free-swimming period. Metamorphosis can be induced by several agents, including larval conditioned seawater, larval tissue-extract (Svane et al., 1987) and heavy metal ions (Whittaker, 1964). With few exceptions, elevated external K^+ in seawater induces settlement or metamorphosis in many invertebrates (reviewed in Woollacott & Hadfield, 1996). Calcium ion has also been reported to be involved in larval settlement and metamorphosis in several marine invertebrates (Freeman & Ridgway, 1987; Ilan et al., 1993; Clare, 1996). In the present study, the effects of K^+ and Ca^{2+} on metamorphosis in the ascidian, *Halocynthia roretzi*, were examined.

Halocynthia roretzi were collected during the spawning season near the Asamushi Marine Biological Station of Tohoku University, Aomori, Japan, and the eggs and sperm were obtained according to Matsumura et al. (1999). The eggs were artificially fertilized with a suspension of non-self sperm, and fertilized eggs were cultured in filtered seawater at 13.2°C. Metamorphosis assays were carried out using artificial seawater (ASW; van't Hoff formula: 460 mM NaCl, 10.1 mM KCl, 9.2 mM $CaCl_2$, 35.9 mM $MgCl_2$, 17.5 mM $MgSO_4$, 10 mM Tris-HCl, pH 8.2) as control and ASW modified by addition or subtraction of various cation concentrations in polystyrene multiwell plates (Corning Cell Wells 25815). Fourteen to 18 tadpole larvae, within 4 h of hatching, were incubated in 4 ml of ASW at various cation concentrations at 13.2°C. After incubation for 8, 24, 48 and 72 h, the plates were observed under a binocular microscope. The rate of metamorphosis was determined by the rate of tail resorption. Each experimental treatment was duplicated within the same batch of larvae, and repeated at least three times using different batches.

Artificial seawater which had been manipulated to give a range of K^+ concentrations was tested for its effect on metamorphosis (Figure 1). In the control (10.1 mM K^+), larvae did not metamorphose during 24 h incubation and less than 20% of larvae had metamorphosed by 72 h. In 20 mM K^+, metamorphosis was promoted markedly; 20% of larvae had metamorphosed by 8 h and almost 100% had metamorphosed by 72 h. Above 40 mM K^+, metamorphosis was promoted in a dose-dependent way; about 60% of larvae completed metamorphosis by 8 h and 100% by 72 h at 50 mM K^+. However, the rate of metamorphosis at 30 mM was extremely low, but almost all larvae continued to swim even after 72 h of incubation.

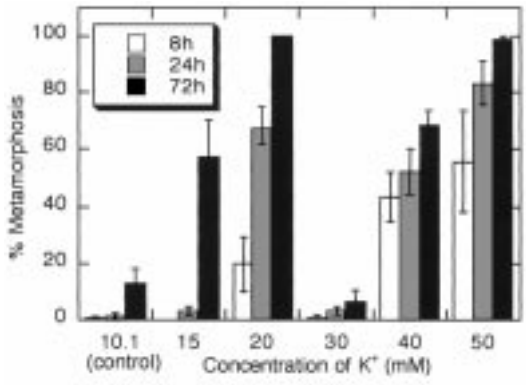

Figure 1. The effect of altering external K^+ concentration on larval metamorphosis in *Halocynthia roretzi*. K^+ concentration of normal van't Hoff formula ASW is 10.1 mM. High K^+ seawater was prepared by replacing NaCl with KCl. Values are mean percentages (±SE) of metamorphosed larvae after 8 (□), 24 (▒) and 72 h (■) incubation from four experiments each using different batches of larvae.

In metamorphosis of various marine invertebrates, K^+ may act by directly depolarizing membrane potential of excitable cells involved in the larval perception of inductive stimuli (Baloun & Morse, 1984). However, the exact mechanism by which potassium affects metamorphosis has yet to be determined (Holm et al., 1998). In *H. roretzi*, 20 mM and 50 mM K^+ induced metamorphosis, but, 30 mM K^+ did not. Woollacott & Hadfield (1996) reported a bimodal pattern in metamorphic response to elevated K^+ in a sponge. Similar bimodal effects of KCl were presented but not discussed in gastropods (Pechenik & Heyman, 1987; Todd et al., 1991), a polychaete (Carpizo-Ituarte & Hadfield, 1998) and echinoids (Pearce & Scheibling, 1994). The possible ubiquity of a dual-phase effect of K^+ has not been remarked upon before, but is supported by the available data. It is possible that more than two types of (i) K^+ channels in the same cell or (ii) of external K^+-dependent excitable cells are involved in the induction of metamorphosis.

A series of ASWs with various Ca^{2+} concentrations was tested (Figure 2A). The tadpole larvae did not swim in Ca^{2+}-free SW (even in the absence of any chelators). At 0.5–5 mM Ca^{2+} metamorphosis was promoted remarkably although abnormal

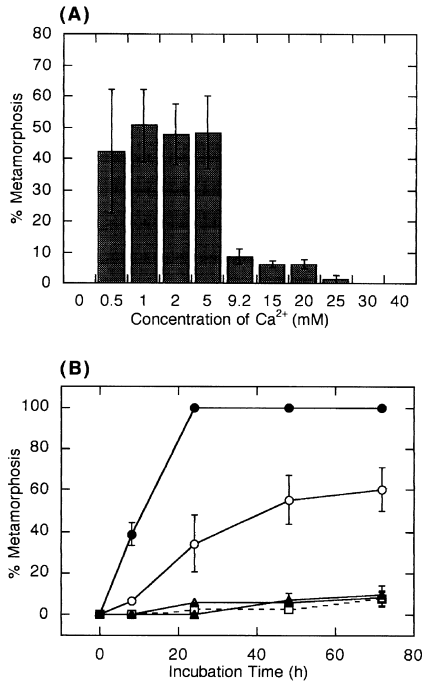

Figure 2. (A) The effect of altering the external Ca^{2+} concentration on larval metamorphosis in *Halocynthia roretzi*. Ca^{2+} concentration of normal ASW is 9.2 mM. Low Ca^{2+} seawater was prepared by decreasing $CaCl_2$. Values are mean percentages (±SE) of metamorphosed larvae after 48 h incubation from three experiments using different batches of larvae. (B) The effect of BAPTA and BAPTA-AM on larval metamorphosis in *H. roretzi*. □, control; △, 1 μM BAPTA; ▲, 10 μM BAPTA; ○, 1 μM BAPTA-AM; ●, 10 μM BAPTA-AM. Values are mean percentages (±SE) of metamorphosed larvae after 8, 24, 48 and 72 h incubation from three experiments using different batches of larvae.

metamorphosis—incomplete resorption of tail and formation of ampullae—was observed at 0.5–1 mM Ca^{2+}. Conversely, high Ca^{2+} concentrations (>25 mM) did not induce metamorphosis; at 30–40 mM, almost no larvae had metamorphosed by 72 h. The motility of larvae also decreased at high Ca^{2+} concentrations (>25 mM).

BAPTA (1,2-bis(2-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid), a calcium chelator, had no effect on the metamorphosis (Figure 2B). BAPTA-acetoxymethyl ester (BAPTA-AM), however, which unlike BAPTA is a cell permeable intracellular Ca^{2+} chelator (Tsien, 1980), was found to promote larval metamorphosis at 1–10 μM. All larvae metamorphosed within 24 h in 10 μM BAPTA-AM.

Excess external Ca^{2+} induced metamorphosis in the larvae of a polychaete (Ilan et al., 1993) and a hydrozoan (Berking, 1988), however, decreased Ca^{2+} inhibited the settlement and metamorphosis of several species (Baloun & Morse, 1984; Rittschof et al., 1986; Berking, 1988; Clare, 1996). In contrast to the above studies, the present study demonstrated that decreased Ca^{2+} (0.5–5 mM in seawater) promoted larval metamorphosis in *H. roretzi* while higher Ca^{2+} (>25 mM) did not. However, Ca^{2+}-free seawater was toxic to the larvae and metamorphosis at 0.5–1 mM Ca^{2+} seawater was abnormal, suggesting that external Ca^{2+} is necessary for normal metamorphosis. Nevertheless, the intracellular Ca^{2+} chelator, BAPTA-AM promoted metamorphosis while BAPTA did not. These results suggest that a decrease in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of certain cells triggered signal transduction involved in metamorphosis (tail resorption) in *H. roretzi*. This is the first report that a decrease in $[Ca^{2+}]_i$ may be important to the induction of larval metamorphosis.

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