Production of tetrodotoxin in puffer fish embryos

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Received 11 March 1998; received in revised form 29 July 1998; accepted 1 September 1998

Abstract

It has been accepted that puffer fish accumulates tetrodotoxin through the food chain. This indicates that tetrodotoxin in puffer fish is exogenous. The present study, however, describes an endogenous origin of tetrodotoxin in puffer fish. For this purpose, the ovulated oocytes from puffer fish Fugu niphobles were artificially fertilized and cultivated. The toxin levels of embryos increased gradually with development until the time of hatching, suggesting that the increased toxin is a product of embryos. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Biosynthesis; Puffer fish; Tetrodotoxin

1. Introduction

Tetrodotoxin is one of the most potent marine toxins. This toxin is predominantly isolated from the ovary and liver of puffer fish (Kao, 1966). However, it has been accepted that the toxin is produced only by various species of bacteria (Yasumoto et al., 1986a, Noguchi et al., 1986, Simidu et al., 1987), and that tetrodotoxin-bearing animals including puffer fish (Noguchi et al., 1982, Miyazawa et al., 1986) may have absorbed and accumulated tetrodotoxin through the food chain (Yasumoto and Yotsu-Yamashita, 1996). The present study, however, shows an endogenous origin of tetrodotoxin in puffer fish. For this purpose, the ovulated oocytes obtained from Fugu niphobles were artificially fertilized and cultivated. As a result, tetrodotoxin levels in the embryos increased with development. This suggested that tetrodotoxin is produced in embryos during development.

2. Materials and methods

2.1. Cultivation of embryos

Artificial sea water was used throughout this experiment. The ovulated oocytes obtained from six litters of F. niphobles females captured in the spawning season were artificially fertilized in each plastic box and then cultured by shaking slowly on a flask shaker until the time of hatching, which was observed on the fifth day after fertilization. To supply the sperm, a spermating male of F. niphobles was caught in the same area. The sperm were dropped into 100 ml of artificial sea water and mixed. The sperm solution was showered onto the ovulated oocytes in each plastic box and mixed. The oocytes were washed immediately several times with artificial sea water and mixed. The sperm solution was showered onto the ovulated oocytes in each plastic box and mixed. The oocytes were washed immediately several times with artificial sea water after fertilization to remove the unfertilized sperm. The water was renewed every day. More than 98% of the fertilized eggs hatched normally.

2.2. Measurement of tetrodotoxin concentration

The tetrodotoxin concentration of embryos and larvae was measured by an indirect competitive enzyme immunoassay using a monoclonal antibody against

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tetrodotoxin as was reported previously (Matsumura, 1995a). In this experiment, 100 embryos or larvae were pooled at the different stages and washed with distilled water. The pooled embryos were homogenized in 0.1% AcOH. The homogenate was boiled for 20 min in a water bath and then centrifuged at 12000 × g. The supernatant was evaporated to dryness. The residue was dissolved in 1 ml of 0.02 M phosphate buffered saline, pH 7.2 (PBS) (equivalent to tetrodotoxin content in 100 embryos per ml). For use in the enzyme immunoassay, the tetrodotoxin solution was diluted optimally with PBS.

3. Results

Tetrodotoxin concentrations of embryos are presented in Fig. 1. After fertilization, toxin levels increased continuously with the embryonic development from day 1 postfertilization. This was observed until the completion of hatching, which occurred day 5 postfertilization. On the other hand, toxin levels in larvae slightly decreased after the hatching compared to those of day 4 (P < 0.05). As a result, the increased volume of tetrodotoxin from day 0 (two cell stage embryos at 3 h after fertilization) to day 4 was 11.2 ng per embryo on average (P < 0.01). These results suggested that the increased tetrodotoxin in this period is a product of embryos. No detectable tetrodotoxin was observed in the cultivation medium (data not shown).

4. Discussion

To demonstrate that puffer fish have an ability to produce tetrodotoxin, the ovulated oocytes obtained from a puffer fish, *F. niphobles*, which is a strongly toxic species (Endo, 1984), were artificially fertilized and cultivated. The toxin levels of embryos increased continuously with development, suggesting that the increased tetrodotoxin during development is a product of the embryos, because it is unlikely that embryos can take in nanogram concentrations of toxin from the cultivation medium. The toxin levels of larvae slightly declined after hatching, probably due to the removal of toxins that were localized in the perivitelline cavity of the embryos. These observations strongly suggest that puffer fish have an ability to produce tetrodotoxin.

The food chain origin of tetrodotoxin has been supported by the following findings (Yasumoto and Yotsu-Yamashita, 1996): (1) many marine bacteria produce tetrodotoxin (Noguchi et al., 1986, Yasumoto et al., 1986b, Simidu et al., 1987); (2) cultured puffer fish have no tetrodotoxin (Matsui et al., 1982), showing that they have no ability to produce tetrodotoxin; (3) tetrodotoxin is detected in starfish (Noguchi et al., 1982) and other marine animals that are the diet of puffer fish. The toxin production by bacteria was contradicted recently (Matsumura, 1995b). No evidence of tetrodotoxin in cultured puffer fish was reported by Matsui et al. (1982) who used a mouse bioassay (Kawabata, 1978) for the detection. However, this was also contradicted recently by the fact that tetrodotoxin could be detected in the fishes by a highly sensitive enzyme immunoassay (Matsumura, 1995a, 1996). The detection of tetrodotoxin in starfish *Astropecten polyacanthus* was reported by Noguchi et al. (1982). This animal has been important to support the food chain origin of tetrodotoxin. The toxin of *A. polyacanthus* was re-examined by a neutralization test using a monoclonal antibody against tetrodotoxin (Matsumura, 1995a). The neutralization curve differed clearly from that of tetrodotoxin derived from *F. niphobles* ovary (Matsumura, 1995b). In addition, this study showed that puffer fish have an ability to produce tetrodotoxin. These facts may therefore require us to revise the food chain origin of tetrodotoxin in puffer fish. However, the mechanism of tetrodotoxin biosynthesis in puffer fish is now being investigated.

Acknowledgements

The author thanks Drs R. End and S. Miyamura for valuable discussion throughout the present study.
References


