

INVOLVEMENT OF THE RETINOID X RECEPTOR IN THE DEVELOPMENT OF IMPOSEX CAUSED BY ORGANOTIN COMPOUNDS IN FEMALE GASTROPODS

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Abstract

We have recently showed that organotins (tributyltin (TBT) and triphenyltin (TPhT)) bind the human retinoid X receptors (hRXRs) with high affinity, and that injection of 9-*cis* retinoic acid (9CRA), the natural ligand of hRXRs into female rock shells (*Thais clavigera*) induces the development of imposex. Cloning of the RXR homologue from *T. clavigera* revealed that the ligand-binding domain of rock shell RXR was very similar to vertebrate RXR and bound to both 9CRA and organotins. These results suggest that RXR plays an important role in inducing the development of imposex in female gastropods. Injections of 3 concentrations of 9CRA in female *T. clavigera* resulted in the development of imposex in a dose-dependent manner. The penis and vas deferens developed in female *T. clavigera* which received 9CRA injections were histologically the same as those in males. Results on RXR gene expression, RXR protein content, immunohistochemical staining with anti-RXR antibody, and on time-course RXR gene expression after exposure to organotins with *T. clavigera* further supported the hypothesis of the involvement of RXR in the development of imposex caused by organotins in female gastropods.

Introduction

The first report on masculinized female gastropod mollusks was made by Blaber⁽¹⁾, describing a penis-like outgrowth behind the right tentacle in spent females of the dog-whelk, *Nucella lapillus* around Plymouth, U.K. The term *imposex*, however, was coined by Smith⁽²⁾ to describe the syndrome of a superimposition of male type genital organs, such as the penis and vas deferens, on female gastropods. Imposex is thought to be irreversible⁽³⁾. Reproductive failure may occur in females with severe imposex, resulting in population decline or even mass extinction^(4,5). In some species, imposex is typically induced by tributyltin (TBT) and triphenyltin (TPT), chemicals released from antifouling paints used on ships and fishing nets⁽⁶⁻¹⁰⁾. Regarding the induction mechanism of imposex, several hypotheses have been proposed and they can be summarized as follows: 1) increased androgen levels, such as testosterone, due to aromatase inhibition by TBT⁽¹¹⁻¹³⁾; 2) inhibition of the excretion of sulfate conjugates of androgens by TBT⁽¹⁴⁾; 3) disturbance of the release of penis morphogenetic/regressive factor from pedal/cerebropleural ganglia by TBT⁽¹⁵⁾; and 4) increase in a neuropeptide, APGWamide level caused by TBT^(16,17). Experimental evidence, however, is weak for these 4 hypotheses. Because of lack of information on the basic biology of mollusks, knowledge of reproductive physiology and/or endocrinology of gastropods has been very limited. Knowledge has been mainly obtained from certain species of Opisthobranchia (e.g., *Aplysia californica*) and Pulmonata (e.g., *Lymnaea stagnalis*); that is several neuropeptides released from the visceral ganglia, cerebral ganglia, or the prostate gland of gastropods (e.g., *A. californica* and *L. stagnalis*) are egg-laying, ovulation, or egg-releasing hormones⁽¹⁸⁻²⁰⁾. Little knowledge of the reproductive physiology and/or endocrinology of Prosobranchia (including Archaeo-, Meso- and Neogastropoda), however, has been obtained. Thus, promotion of the reproductive physiology and/or endocrinology of Prosobranchia is needed to elucidate the mode of actions of TBT and/or TPT in the development of imposex in gastropods.

Materials and Methods

Laboratory experiments with wild rock shell populations were conducted, using several techniques of molecular biology, biochemistry, histopathology, immunohistochemistry, analytical chemistry and ecotoxicology.

Results and Discussion

It was confirmed that organotins (tributyltin (TBT) and triphenyltin (TPhT)) bind the human retinoid X receptors (hRXRs) with high affinity, and that injection of 9-*cis* retinoic acid (9CRA), the natural ligand of hRXRs into female rock shells (*Thais clavigera*) induces the development of imposex⁽²¹⁾. Cloning of the RXR homologue from *T. clavigera* revealed that the ligand-binding domain of rock shell RXR is very similar to vertebrate RXR and binds to both 9CRA and organotins⁽²¹⁾. These results strongly suggest that RXR plays an important role in inducing the development of imposex in female gastropods. A single-injection of 3 concentrations of 9CRA in female *T. clavigera* resulted in the development of imposex in a dose-dependent manner. The penis and vas deferens developed in female *T. clavigera* which received 9CRA injections were histologically the same as those in males. Results on RXR gene expression, RXR protein content, immunohistochemical staining with anti-RXR antibody, and on time-course RXR gene expression after exposure to organotins with *T. clavigera* further supported the hypothesis of the involvement of RXR in the development of imposex caused by organotins in female gastropods.

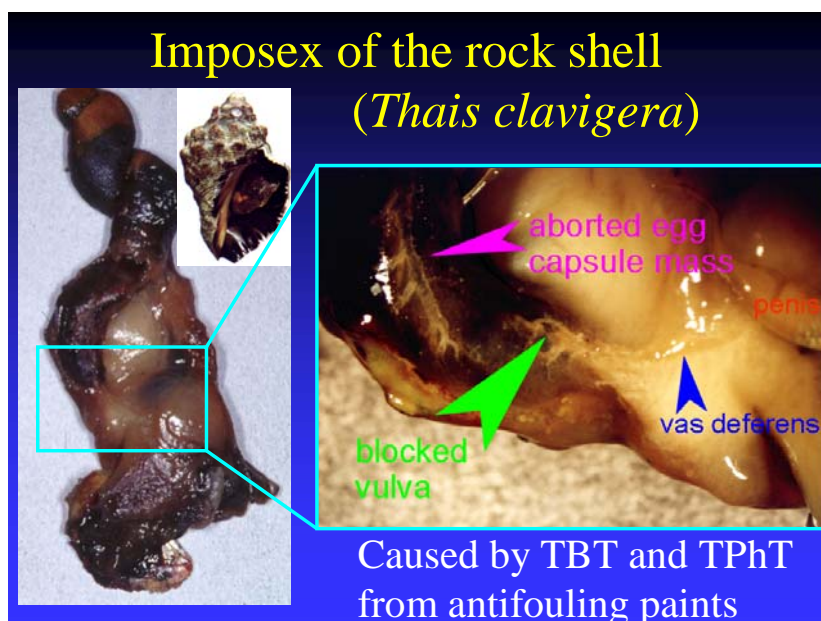
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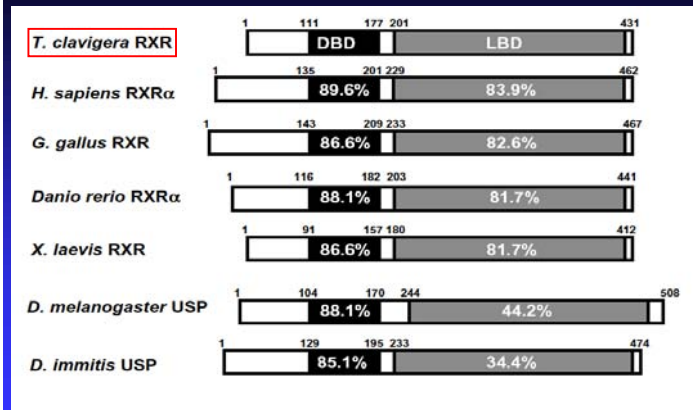
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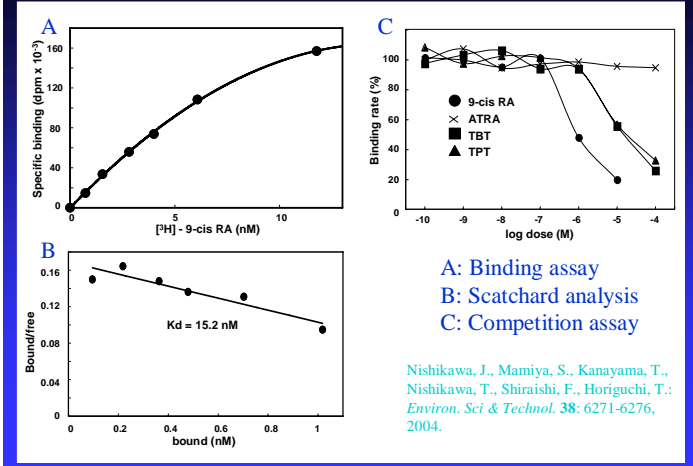


Homology of RXR among Species



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In Vitro Assays with Rock Shell RXR



Effects of 9CRA to the Development of Imposex in *Thais clavigera* (1)

