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Cellular effects of tributyltin (TBT) on the penis epithelium cells of prosobranchs (*Hinia reticulata* and *Ocinebrina aciculata*)

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ABSTRACT: Cytopathological effects on organelles of penis epithelium cells were investigated in prosobranchs that had been exposed for two weeks to three months to high TBT-concentrations in artificial seawater. TBT exposure damaged cell organelles, such as mitochondria, Golgi dictyosomes, endoplasmatic reticulum, and injured the cell membranes. In addition, atypical intercellular spaces were observed between the cells of the epithelial layer. Further cell alterations included the increase of residual bodies within the cells as well as structural changes of the basal lamina. The ultrastructural changes were compared with cell alterations of specimens which had been collected in a polluted environment on the coast of Brittany (France).

INTRODUCTION

Tributyltin which is used worldwide as a biocide in antifouling paints for ships induces a variety of malformations in marine and also in freshwater gastropods. Some of the most important effects of TBT are the imposex and intersex phenomena in prosobranchs (neo- and mesogastropods) which became evident in the 1980s (Blaber, 1970; Gibbs & Bryan, 1986; Fioroni et al., 1991a; Oehlmann, 1994; Schulte-Oehlmann et al., 1995; Stroben, 1994). Imposex is a superimposition of male sex organs (penis and/or vas deferens) on female individuals (Smith, 1971). In highly TBT-polluted areas, especially harbours and marinas, females exhibit malformations of the pallial oviduct which inhibit copulation and spawning, resulting in sterilization. Intensive research on this topic has been carried out during the past few years. Gibbs et al. (1987) discovered that very low levels of TBT (below 1 ng TBT Sn l^{-1}) induces imposex in *Nucella lapillus*, a highly TBT-sensitive neogastropod. On the other hand, intersex which has been observed previously in the periwinkle *Littorina littorea* (mesogastropod) is characterized by disturbance of the congruity between gonad and genital tract. In contrast to imposex, no superimposition of male characters occurs during intersex development, but the organs of the pallial oviduct are modified towards a male morphological structure (Bauer et al., 1995). Most of the studies are based on morphological and histological analyses (Bailey & Davies, 1989; Fioroni et al., 1990, 1991b; Gibbs et al., 1987, 1990, 1991; Oehlmann et al., 1991; Stroben et al., 1992a, b, c). The objective of this report is a detailed description of TBT-induced cellular damage. In former studies we described the fine structure of the penis epithelium of prosobranchs collected from TBT-polluted areas on the coast of Brittany (Brick & Bolte,

1994; Brick & Deutsch, 1993; Deutsch & Brick, 1993). Conscious of the fact that field individuals are intoxicated not only by TBT but also by other environmental toxicants, such as compounds of Hg, Pb, Cd, PCB, we started laboratory experiments with snails which were exposed only to artificial seawater with a constant high TBT-concentration (0.1 μg TBT-Sn l^{-1}). The results of the experiments on these laboratory test animals were compared with those on prosobranchs from polluted fields. Basing on this comparison, we were able to differentiate between TBT-induced cell damage of the laboratory animals and cellular alterations of the field specimens that could have been caused by other environmental pollutants.

MATERIAL AND METHODS

Specimens of *Hinia reticulata* (Buccinidae) and *Ocinebrina aciculata* (Muricidae) were collected from the coast of Brittany (Roscoff/France) and exposed for two weeks (*O. aciculata*) to three months (*H. reticulata*) to artificial seawater containing 0.1 μg TBT-Sn l^{-1} (TBT-chloride, Riedel de Haen AG, Seelze) (salinity: 35 ‰; temperature: 10 °C; specific gravity: 1.027). Control animals were kept in artificial seawater without TBT contamination during the experiment period. After the exposure period, specimens were narcotized in 7 % $MgCl_2$ solution and the shells were cracked with a vice. The penis tissues of the male individuals were immediately fixed in 5 % $K_2Cr_2O_7$ (pH: 7.2–7.4), followed by fixation with 2 % OsO_4 at 4 °C. Subsequently, the samples were dehydrated via graded ethanol series and embedded in Spurr's resin (Spurr, 1969). Sections were cut with a Reichert Ultracut and poststained with 1 % lead citrate for analysis with a Zeiss Electron Microscope (EM 900) at 80 kV.

RESULTS

The ultrastructural organization of the normal outer epithelial cells lining the penis has been described for prosobranchs by Amor (1988, 1990), Brick & Bolte (1994), Brick & Deutsch (1993) and Deutsch & Brick (1993). Electron microscopical analysis revealed that the ultrastructure and organization of the epithelial cells in *Hinia reticulata* were seriously affected by TBT. Remarkable changes in the cell organelles and in the structure of the cytoplasm were observed after a three-month TBT exposure. Wide basal and lateral intercellular spaces were characteristic for the epithelial layer of the TBT-exposed prosobranchs *H. reticulata* and *Ocinebrina aciculata* (Fig. 1a, b). Atypical spaces were also formed between the basal lamina and the basal cell membrane (Fig. 1c). The cell membranes were sometimes incorporated in degenerating processes. In contrast to the control snails, which were not exposed to TBT during the experiment period, the nuclear envelopes were smooth and not invaginated. Further TBT-effects were indicated by dilated Golgi dictyosomes (Fig. 1d) and a conspicuous swelling of the ER-cisternae (Fig. 1e). Smooth ER which plays an important role in detoxification processes could often be detected in the cells (Fig. 1g). Lysosomes as well as residual bodies were also present to a high extent (Fig. 1g, h); but, above all, the mitochondria were damaged by TBT. They were extremely swollen and their matrix was structureless and transparent. The cristae showed an atypical arrangement of parallel stacks or concentric figures (Fig. 1h). In contrast to the controls, the basal laminae were significantly thicker in TBT-exposed specimens of *H. reticulata*.

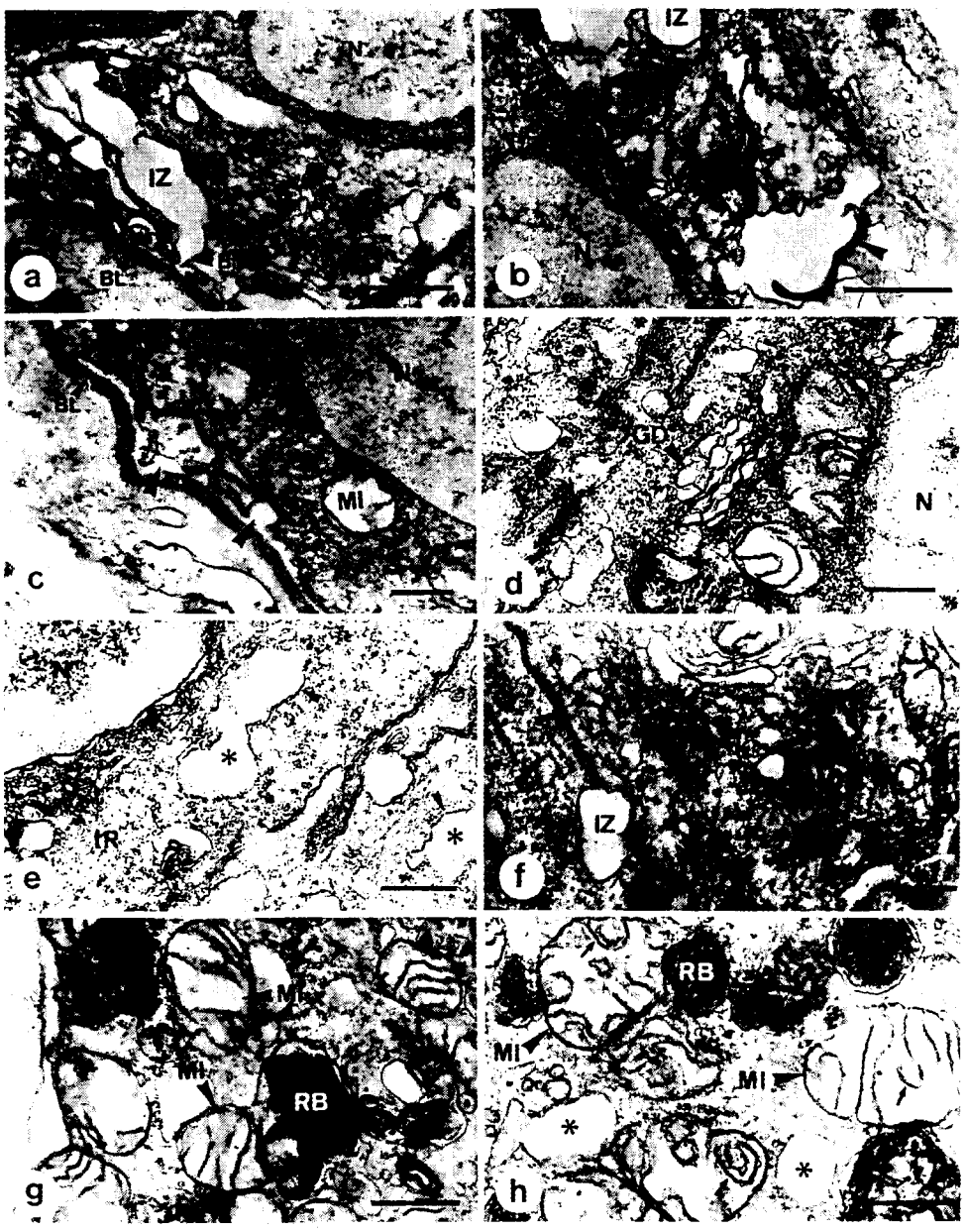

Fig. 1. Cells of the penis epithelium of *Hinia reticulata* after TBT exposure for three months. **a:** Intercellular space in the basal region of the cell; scale bar: 1 µm. **b:** Degenerative membrane processes (arrows); scale bar: 1 µm. **c:** Dilated space between the basal lamina and basement membrane (arrows); scale bar: 0.5 µm. **d:** Dilated Golgi dictyosomes with a loss of polarity; scale bar: 0.5 µm. **e:** Wide dilated ER-cisternae (asterisks); scale bar: 0.5 µm. **f:** High content of smooth ER within the cells; scale bar: 0.5 µm. **g** and **h:** Residual bodies and distinctly damaged mitochondria with atypically arranged cristae (small arrows), abnormal intracellular spaces (asterisks); scale bar: 0.5 µm. BL, basal lamina; BM, basement membrane; fR, free ribosomes; GD, Golgi dictyosomes; MI, mitochondrium; N, nucleus; IZ, intercellular space; RB, residual bodies; sER, smooth endoplasmatic reticulum

Fig. 2. Cells of the penis epithelium of *Ocinebrina aciculata* after TBT exposure for 14 days.
a: Apical view of a cell, containing numerous ribosomes (asterisks, arrows); desmosomes and septated junctions are well developed; scale bar: 0.5 µm. **b:** Dilated rER; scale bar: 0.5 µm. **c:** Dilated cisternae of sER; scale bar: 0.5 µm. **d:** Loss of electron density of the pigment granules after 14 days of TBT exposure in contrast to (**e**) electron-dense pigment granules after 7 days of exposure; scale bar: 0.5 µm. **f:** Well developed mitochondria, sometimes containing small granules (arrows); scale bar: 0.5 µm. **g:** Atypically formed mitochondrium; scale bar: 0.5 µm. **h:** Wide abnormal spaces in the basal cell area; scale bar: 0.5 µm. **i:** Irregularly formed basal lamina with "hole"-like structures in the matrix (white arrow); scale bar: 2 µm. BL, basal lamina; D, desmosome; GD, Golgi dictyosomes; IZ, intercellular space; MI, mitochondrium; MV, microvilli; N, nucleus; PI, pigment granule; rER, rough ER; sER, smooth ER; SJ, septated junction

In *O. aciculata* exposed for two weeks to TBT, organelles which have a function in anabolic processes, such as polysomes, smooth and rough ER, characterized the epithelium cells (Fig. 2). The ER-cisternae were sometimes slightly dilated (Fig. 2b). Swelling of the Golgi dictyosomes was not observed in *O. aciculata*. Septated junctions between the lateral cell membranes were well developed (Fig. 2a). Pigment granules which are a common feature within the cells of prosobranchs lack electron density in contrast to the control specimens (Fig. 2d, e). Unlike the mitochondria of *H. reticulata*, those of *O. aciculata* were compact and well developed (Fig. 2e, f), sometimes containing granules within the matrix (Fig. 2e). Besides normal mitochondria, some atypically formed mitochondria were observed (Fig. 2g). Wide intercellular spaces were detected within the epithelial layer (Fig. 2h). The basal lamina showed "hole"-like structures (Fig. 2i) which have been described in *Buccinum undatum* from TBT-polluted areas (Brick & Bolte, 1994).

DISCUSSION

Tributyltin induces a wide range of changes on the ultrastructural level in penis epithelial cells of prosobranchs. A characteristic of TBT cell toxicity is a high incidence of damage to mitochondria. Bodar et al. (1990), Viarengo (1989) and Yoshizuka et al. (1992) discovered a high vulnerability in mitochondrial membranes towards organotins and other heavy metals. TBT inhibits the uptake of oxygen and forestalls the synthesis of ATP by inhibiting the oxidative phosphorylation (Brierley, 1977; Wester et al., 1990; Widdows & Page, 1993).

Wide atypical intercellular spaces, which were not observed in the control specimens, seem to be indicative of damage to the cell membranes in *Hinia reticulata* and *Ocinebrina aciculata*. Similar structures have been described by Elendt & Storch (1990) in the epithelium of the digestive gland of *Daphnia* after a period of starvation stress. We, too, have never observed any feeding activity in TBT-exposed snails, in contrast to the controls in our experiments. We suppose that the spaces were probably formed through TBT-induced starvation stress. Dilated ER and a high content of smooth ER and lysosomes were indicative of detoxification processes in the cells (Bayne et al., 1985; Brauneck et al., 1989; Burkhardt-Holm et al., 1990; Fowler, 1987; Moore, 1985). Rubio et al. (1993) supposed that dilated ER and Golgi dictyosomes are important for efficient proteinsynthesis, mainly for the synthesis of metalloproteins which play a role in detoxification mechanisms. Bleaching of the pigment granules, which was observed in *O. aciculata*, has also been described by Fent (1992) and Fent & Meier (1992) in TBT-exposed freshwater fish *Phoxinus phoxinus*. Atypical mitochondria and dilated ER as well as Golgi dictyosomes were common in both field and laboratory specimens; but we identified a major destruction of cells and organelles in the epithelium of polluted field specimens. In imposex females collected from the field we could observe additional epithelial disruption of the basal lamina. Furthermore, we noticed in these snails that the epithelium cells penetrated through the basal lamina into the muscle tissue underneath (Brick & Deutsch, 1993). These two phenomena were not observed in prosobranchs in laboratory tests. In conclusion, similar cell alterations like swelling of mitochondria, dilated ER and Golgi dictyosomes, increase of lysosomes and residual bodies can be established in field and TBT-exposed laboratory specimens. It must be stated, however, that the cells of imposex-affected females from the field showed a higher extent of cell damage than the laboratory

test specimens, probably because of several environmental xenobiotics. In summary, the main effects of TBT are (a) damage of organelles that are important for cell metabolism and (b) reduction of the cell integrity within the epithelium layer.

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