Genotoxic effects of anoxia on *Mytilus galloprovincialis*

R. Brunetti, O. Fumagalli, P. Valerio, M. Gabriele

Department of Biology, University of Padova, Via Trieste 75, I-35121 Padova, Italy

ABSTRACT: In mussels *Mytilus galloprovincialis* Lmk. exposed to the air or kept in oxygen-free seawater, the frequency of micronuclei detected in gill tissues significantly rises as a function of time. It is suggested that the genotoxic agent may be a product of anaerobic metabolism, and the genotoxicity of propionic and acetic acid are shown, although the possibility that other substances may be involved in the phenomenon is not excluded.

INTRODUCTION

In the marine environment anoxia is a phenomenon generally localized though not rare. Bottom-living marine animals are subjected to hypoxic or anoxic conditions as a consequence of particular hydrodynamic conditions or eutrophication of waters. Anoxia is of course recurrent in animals living in the intertidal zone. Mussels react to exposure to air or to reduced oxygen contents of waters by closing their valves and changing to anaerobic metabolism (De Zwaan & Wijman 1976, Gade 1983).

In a previous study on the micronucleus test in *Mytilus galloprovincialis* Lmk. we observed an unexpectedly high frequency of micronuclei in gill tissues of mussels kept in air either at room temperature or in the cold, suggesting that this effect may be due to the genotoxic activity of some catabolic product of their anaerobic metabolism (Brunetti et al. 1988). In fact micronuclei, which are small chromatid bodies formed during telophase near the principal nucleus as a consequence of chromosome breakage or loss of an entire chromosome during anaphase, may be used as an index of chromosome damage (= genotoxicity) (Heddle et al. 1983, Keith & Balder 1989).

In the present study we confirm our previous observations showing that in anoxic mussels the micronucleus frequency rises. Moreover, since the most important end-products of the anaerobic metabolism of *Mytilus* spp. are propionic and acetic acid (De Zwaan 1977, Van Den Thillart & De Vries 1985), we tested the micronucleus-inducing ability of these 2 carboxylic acids.

MATERIALS AND METHODS

Mussels for experiments were collected in different seasons from cultures in the Venetian Lagoon, Italy, cleared of epibionts, and transferred within 1 h to laboratory aquaria at the same temperature as the natural waters. In each set of experiments mussels of the same age and approximately the same size were used.

**Exposure to air at different temperatures.** In these experiments the effects of air exposure at 2 temperatures (5 and 25 °C) on micronucleus frequencies were tested on sexually ripe (October 1988 & February 1989) and unripe (June 1989) mussels.

**Exposure to air at different temperatures and anoxic water.** To study the effects of air exposure and anoxic water at the same temperature at which the mussels were collected, 2 experiments were performed at 10 and 25 °C with ripe (March 1990) and unripe (June 1990) mussels. Effects of air exposure were also tested on mussels whose valves were tied to avoid any exchange with the atmosphere, since it has been reported that some mussel species open their valves during exposure to air, suggesting some use of atmospheric oxygen (Bayne et al. 1976b, Ahmad & Chaplin 1977). A slight degree of shell gape was reported in *Mytilus edulis* (Coleman 1973) and *M. californianus* (Bayne et al. 1976a), and was also observed by us in *M. galloprovincialis*.

Experiments in anoxic water were performed in sealed 500 ml vials filled with filtered water, with only 1 mussel per vial. The oxygen content of the water was reduced by flushing with nitrogen and was checked at...
the beginning and end of experiments with an oximeter. Dissolved oxygen ranged from 0.5 to 0.8 ppm. Lower values were discarded to avoid the formation of hydrogen sulphide.

**Exposure to propionic and acetic acids.** Treatments with propionic and/or acetic acid were performed in glass aquaria, and the conditioned water was renewed every 6 h. Concentrations of propionic acid used were 4 and 0.3 mM 1⁻¹ and for acetic acid 2 and 0.1 mM 1⁻¹. Length of treatment was either 6, 48 or 72 h. Genotoxic effects were detected by means of the micronucleus test (Heddle et al. 1983). According to the technique, more extensively described in Brunetti et al. (1992) (this issue), the gills were removed from mussels 0 or 2 and 10 d after the end of treatment, enzymatically digested and the obtained cell-suspension fixed with methanol:acetic acid (3:1) and centrifuged. The resulting pellets were spread on slides, air-dried and stained with 5 % Giemsa. Two thousand cells per mussel were scored.

**RESULTS AND DISCUSSION**

**Effects of air exposure**

Effects of air exposure at 2 temperatures on micronucleus frequencies are shown in Fig. 1. In all cases a linear increase as a function of time was observed, although in the June 1989 mussels exposed to air at low temperature this increase occurred after only 3 d of treatment (Fig. 1C). This may be due to a temporary metabolic pause as a consequence of the sudden passage from the high summer environmental temperature (about 25 °C) to the cold experimental one (5 °C).

Fig. 1 also indicates that induced micronucleus frequencies rapidly fell to control values when mussels were immersed in water at 15 °C.

**Effects of air exposure and anoxic water**

Results of changes in air temperature and of anoxic water experiments are shown in Fig. 2. Responses of ripe and unripe mussels at the 3 experimental conditions did not differ, although the increase in micronucleus frequencies was more rapid at 25 °C. A statistically significant difference from controls was noted on Days 3 & 4 of treatment at 25 and 10 °C respectively.

These results are not in agreement with the concept that in periods of oxygen-deficiency mussel metabolism falls at a 'standard' temperature-independent rate (Newell & Northcroft 1967, Coleman 1973). In fact, in anoxic individuals anaerobic metabolism may be briefly involved, nullifying the meaning of oxygen uptake in evaluating energy demands (De Zwaan & Wijsman 1976).

A trend towards diversification among the 3 experimental conditions as a function of time was also noted. This may indicate easier removal of the genotoxic agent in air-exposed mussels with free valves or a lower accumulation of toxic agents as a consequence of the aerial rate of oxygen uptake (Widdows et al. 1979).

**Effects of propionic and acetic acids**

This set of experiments was performed to test the hypothesis that the genotoxic agent(s) acting during anoxia may be propionic and/or acetic acid, which are the most important end-products of anaerobic metabolism in these mussels (De Zwaan 1977).

Table 1 shows the results of the 3 preliminary experiments. The concentrations tested in Expts 2 & 3 were chosen on the basis of the study of Van Den Thillart & De Vries (1985), who found 0.3 and 0.1 mmol of propionic and acetic acids respectively in the water sur-

Fig. 1. *Mytilus galloprovincialis*. Ordinate: mean micronuclei frequency (%) induced by air exposure at 5 and 25 °C in ripe (A: October 1988; B: February 1989) and unripe (C: June 1989) mussels; abscissa: time in days. Vertical bars: 95 % confidence limit of mean (10 mussels per experimental point, 2000 cells ind⁻¹). Dashed lines: pattern of frequencies after reimmersion. Statistically significant differences from controls (c): ** = p < 0.01, *** = p < 0.001)
Fig. 2. *Mytilus galloprovincialis*. Ordinate: mean micronuclei frequency (%) induced in air-exposed mussels with free (fv) and tied (tv) valves and in individuals kept in anoxic water (aw). A: Ripe mussels (March 1990); B: unripe mussels (June 1990); abscissa: time in days. Vertical bars: 95% confidence limit of mean (10 mussels per experimental point, 2000 cells ind.−1). Statistically significant differences from controls (c): (** = p < 0.01; *** = p < 0.001).

These data indicate that both substances are genotoxic. Moreover, because the ratio of the increase of mean micronucleus frequencies induced by propionic and acetic acids compared to that of controls is about 1.4, and the ratio of the contration of propionic acid and acetic acid used was about 3, it is inferred that acetic acid is more toxic.

During treatments specimens maintained apparently normal rates of filtration and defecation. However, after 6 h of treatment at the high acid concentrations of Expt 1 the mussels showed an excessive degree of shell gape and no reaction to tactile stimuli. When transferred to unconditioned seawater, mussels returned to normality in a few hours. Ten days after the end of Expt 1, only mussels previously treated with propionic acid had micronucleus frequencies significantly higher than those of controls.

Fig. 3A, B shows the results of 2 other experiments performed on (A) unripe and (B) ripe mussels at the same environmental temperature at which the animals had been collected. These data indicate the probable additive effect of the 2 acids when used together.

A substantial difference was also observed between the 2 experiments. In (A), micronucleus frequencies mainly rose after the end of treatment, while in (B) the highest increase took place in the presence of the acids and, at least for micronuclei induced by propionic acid, decay immediately occurred after the end of treatment. At present, no elements allow us to infer whether this

| Sample size = 10; 2000 cells ind.−1 |

Table 1. *Mytilus galloprovincialis*. Micronuclei frequency (× 10^3) induced by acetic and propionic acids. T: time (d) from end of treatment; \( \bar{x} \): mean; SD: standard deviation; p: statistical comparison with controls; * p < 0.5, *** p < 0.001, ns: not significant.

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<tr>
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<th>Expt 1 6 h treatment</th>
<th>Expt 2 48 h treatment</th>
<th>Expt 3 72 h treatment</th>
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<td>Propionic acid conc.</td>
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<td>4.14  2.09 ***</td>
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<td>0.3 mM 1^{-1}</td>
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<td>1.96  0.96'</td>
<td>4.25  1.63 ***</td>
<td>4.66  1.68 ***</td>
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<tr>
<td>Acetic acid conc.</td>
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<tr>
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<td>2.14  1.35 ***</td>
<td>0.1 mM 1^{-1}</td>
<td>0.1 mM 1^{-1}</td>
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<tr>
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<td>3.35  1.75 ***</td>
<td>3.52  0.87 ***</td>
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the genotoxic agent rather than a slower mitotic rate occurring in the former.

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LITERATURE CITED


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