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In vitro and in vivo inhibition of *Daphnia magna* acetylcholinesterase by surfactant agents: possible implications for contamination biomonitoring

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Abstract

This study was designed to investigate the effect of two surfactants, dodecyl benzyl sulfonate (DBS), sodium dodecyl sulfate (SDS), and of a domestic detergent (Y) on the AChE activity of the crustacean cladoceran *Daphnia magna*. All the chemicals significantly inhibit the activity of the enzyme, both in vitro and in vivo conditions. In vitro lowest observed effect concentration (LOEC) values ranged from 12.5 to 100 mg/l and correspondent IC_{50} (50% inhibition concentration) values ranged from 6.6 to 58.5 mg/l. In vivo LOEC values ranged from 2 to 11.9 mg/l, while EC_{50} (50% effect concentration) values ranged from 11.4 to 56.7 mg/l. AChE inhibition by environmental contaminants such as surfactants, detergents and metals may lead to false diagnostics and even wrong conclusions in biomonitoring studies based on the use of AChE as a specific biomarker for organophosphorous and carbamate compounds.

Keywords: Biomarkers; Acetylcholinesterase; *Daphnia magna*; Surfactants

1. Introduction

Acetylcholinesterase (AChE) is the enzyme that hydrolyses the neurotransmitter acetylcholine in cholynergic synapses of both vertebrates and invertebrates. This enzyme is strongly inhibited by organophosphate and carbamate pesticides at low concentrations and, for this reason, has been widely used as a specific biomarker for these compounds.

In the last decades, the inhibition of cholinesterases from several species by environmental contaminants other than organophosphate and carbamate pesticides has been increasingly reported. The group of metals was, probably, the first class of environmental pollutants that was found to include non-specific anti-cholinesterase agents. The potential of some metallic ions, such as Hg^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} to depress the activity of cholinesterases of fish and inverte-

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brates, in vitro and/or in vivo conditions, has been demonstrated in several studies (Olson and Christensen, 1980; Gill et al., 1990, 1991; Schmidt and Ibrahim, 1994; Labrot et al., 1996). Recently, undetermined components of complex mixtures of pollutants were also found to inhibit AChE activity (Payne et al., 1996). Synthetic detergents are major pollutants of the aquatic environment. These compounds have both industrial and domestic uses and are released into natural waters in effluents. Modern detergents are biodegradable in water and are considered not to accumulate in the environment (Malcom et al., 1995). Nevertheless, due to a continuous input they may attain relatively high concentrations in some areas (WHO, 1996). Studies published in the last years report in vitro inhibitor effects of detergents and surfactants on the activity of cholinesterases of *Mytilus galloprovincialis* and *Moina macropa* (Martínez-Tabche et al., 1996; Guilhermino et al., 1998) at concentrations in the range of milligrams per litre.

Daphnia magna is a standard organism in ecotoxicology. It is relatively easy to maintain in the laboratory, has a short life cycle, requires low culture and test medium volumes and a great deal of information available about it exists. Furthermore, the inhibition of *D. magna* AChE is a suitable criterion to be used in toxicity tests (Guilhermino et al., 1996a). For these reasons, *D. magna* was chosen as the test organism in this study.

Here, we investigated the in vitro and in vivo effects of two surfactants, dodecyl benzyl sulfonate (DBS) and sodium dodecyl sulfate, and of a mixture commonly used as domestic detergent (Y) on the activity of the enzyme acetylcholinesterase of *D. magna*.

2. Material and methods

2.1. Parental cultures

Progenitors were cultured in groups of 10 per 800 ml of the medium ASTM (American Society for Testing and Materials) hard water (ASTM, 1980) with an organic additive (Baird et al., 1989) and fed three times a week with 0.322 mg of Carbon per daphnia per day. Photoperiod was 16:8 h L/D and temperature was 20 ± 1°C.

2.2. Toxicity tests

In vitro and in vivo AChE inhibition tests were performed according to Guilhermino et al. (1996a). Briefly, for in vitro tests, juveniles (< 24 h old) were isolated from parental cultures and maintained in groups of 20 per 800 ml of ASTM during 48 h with no food. After this period, organisms were used to prepare homogenates which were incubated during 30 min with several concentrations of each toxicant (0, 12.5, 25, 50 and 100 mg/l). In vivo AChE inhibition tests were performed by exposing juveniles (< 24 h old) to several concentrations of each test substance (DBS: 0, 2, 3, 4.5, 6.7 and 10 mg/l; SDS and Y: 0, 7.9, 11.9, 17.8, 26.7 and 40 mg/l) for 48 h. After this period, live animals were used to prepare homogenates for AChE determinations.

2.3. Homogenate preparation

Homogenates were prepared in ice cold phosphate buffer (0.1 M, pH 7.2) using a homogeniser, according to the procedure described by Guilhermino et al. (1996b).

2.4. AChE determination

AChE activity in the homogenates was determined by the method of Ellman et al. (1961), adapted to microplate (Herbert et al., 1995) and optimised for *D. magna* (Guilhermino et al., 1996b). Acetylthiocholine was used as substrate in all the assays and no attempt was made to distinguish between AChE and pseudo-cholinesterase. The activity of the enzyme was expressed in units (U) per milligrams of protein, corresponding 1 U to 1 nmol of substrate hydrolysed per minute per millilitre. A Labsystems Multiskan MS microplate reader was used.

2.5. Protein concentration

Protein concentration in the samples was de-

termined by the Bradford technique (Bradford, 1976) and adapted to microplate (Herbert et al., 1995).

2.6. Toxicant solutions

Solutions of DBS, SDS and Y were prepared immediately before the beginning of the tests in micro-pure water. For each test, daily prepared solutions were used. Data analysis was based on nominal concentrations.

2.7. Chemicals

Acetylthiocholine, 5,5′-dithio-bis(γ-nitrobenzoic acid) (DTNB) and γ-bovine globulins were purchased from SIGMA (USA), Bradford reagent was from BIO-RAD (UK). DBS (> 98% pure) was a gift from UNILEVER (UK), SDS (99% pure) was from MERCK (Germany). 'Y' was a washing mixture for domestic use commonly found in the market.

2.8. Data analysis

Data were analysed by Analysis of Variance (ANOVA). No-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values were determined by Ducan multicomparison test. The concentration of inhibition at 50% (IC_{50}) and the 50% effect concentration (EC_{50}) values were calculated by probit analysis (Finney, 1971). The significance level was 0.05.

3. Results

All the compounds significantly inhibited the AChE activity of *D. magna* in vitro (DBS: $F = 31.6$, $P < 0.05$; SDS: $F = 102.3$, $P < 0.05$; Y: $F = 16.3$, $P < 0.05$) (Fig. 1). DBS was the more effective inhibitor [NOEC < 12.5 mg/l, LOEC = 12.5 mg/l and $IC_{50} = 6.6$ mg/l (95% CL = 6.1–7.2 mg/l)] and Y was the least effective [NOEC = 50 mg/l, LOEC = 100 mg/l and $IC_{50} = 58.8$ mg/l (95% CL = 52.1–65.5 mg/l)]. SDS showed an anti-cholinesterase intermediate effect [NOEC = 25 mg/l, LOEC = 50 mg/l, $IC_{50} = 50.1$ mg/l (95% CL = 44.2–56.9 mg/l)].

Daphnids exposed in vivo to DBS, SDS or Y showed lower levels of AChE than unexposed animals (Fig. 2). To each chemical, the differences among treatments are significant (DBS: $F = 18.7$, $P < 0.05$; SDS: $F = 13.5$, $P < 0.05$; Y: $F = 39.2$, $P < 0.05$). DBS significantly depressed AChE activity at concentrations equal to, or higher than

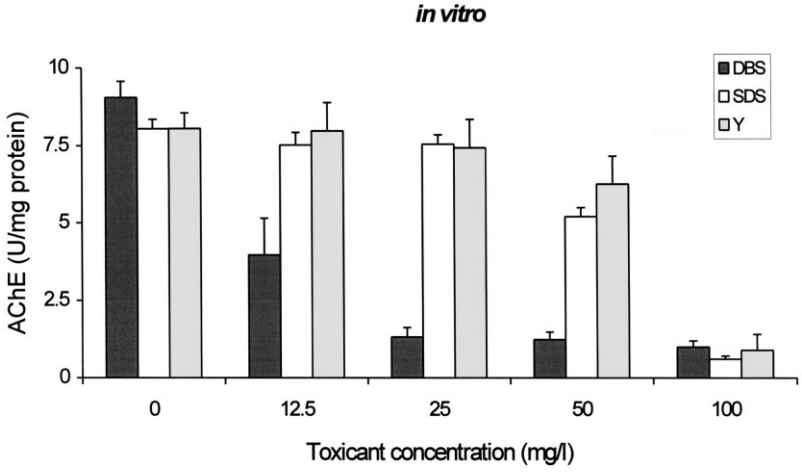

Fig. 1. In vitro effect of DBS, SDS and Y on AChE activity of *D. magna*. Values are the mean of three replicates with correspondent 95% error bars (S.E.M.).

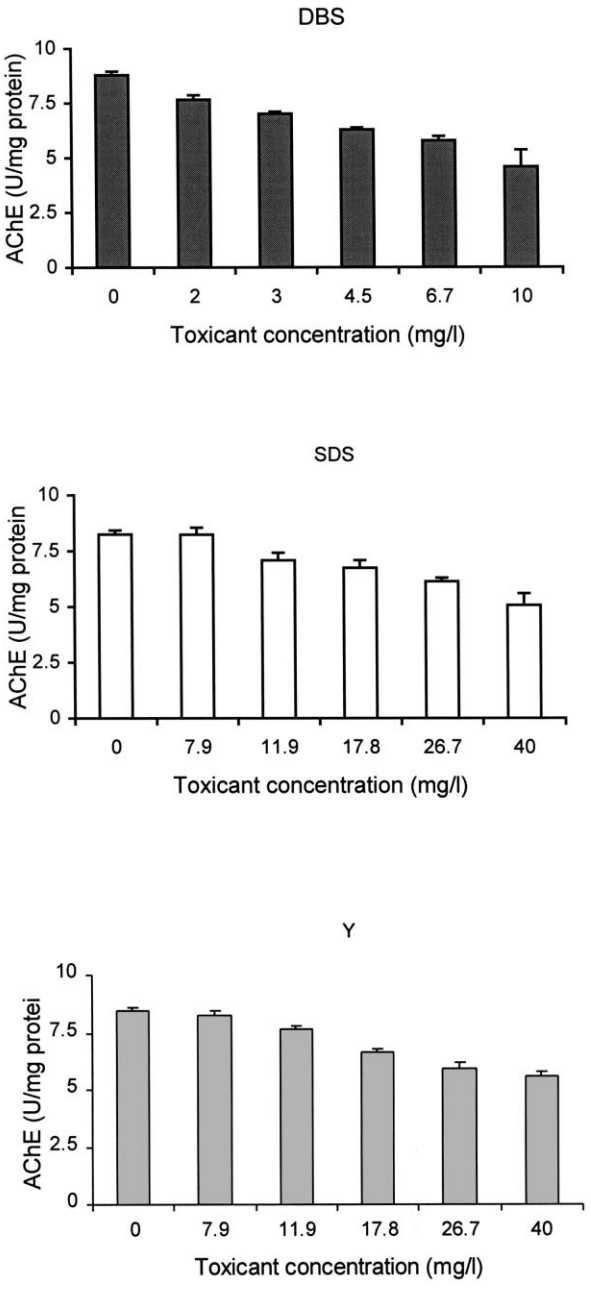

Fig. 2. In vivo effect of DBS, SDS and Y on AChE activity of *D. magna*. Values are the mean of three replicates with correspondent 95% error bars (S.E.M.).

2 mg/l (NOEC < 2 mg/l), SDS at concentrations equal to, or higher than 11.9 mg/l (NOEC = 7.9 mg/l) and Y at concentrations equal to, or higher than 17.8 mg/l (NOEC = 11.9 mg/l). EC_{50} values were 11.4 mg/l (95% CL = 8.1–15.9 mg/l) for DBS, 51.5 mg/l (95% CL = 50.6–52.3 mg/l) for SDS and 56.7 mg/l (55.4–58.2 mg/l) for Y.

4. Discussion

This study was designed to investigate the effect of DBS, SDS and Y on AChE activity of *D. magna*. All the tested chemicals significantly inhibited the activity of the enzyme, both in vitro and in vivo conditions. These results are in complete agreement with other studies reporting in vitro anti-cholinesterase effects of surfactants (Martínez-Tabche et al., 1996; Guilhermino et al., 1998).

In vitro LOEC (50 and 100 mg/l) and IC_{50} (51.5 and 56.7 mg/l) values found in this study for SDS and Y are higher than the range of surfactant concentrations (individual chemical concentrations) reported in the literature as generally occurring in aquatic environments. However, an in vitro LOEC of 12.5 mg/l and an IC_{50} of 6.6 mg/l were found for DBS. These values compare with surfactant concentrations measured in polluted surface waters (1–10 mg/l) (WHO, 1996). Furthermore, in vivo LOECs were 2 mg/l for DBS and 11.9 mg/l for SDS and Y, while EC_{50} values for these compounds were 11.4, 51.5 and 56.7 mg/l, respectively. Concentrations of linear alkylbenzene sulfonates (LAS) near 10 mg/l have been measured in effluents (WHO, 1996). Total amount of surfactants and detergents in these effluents was probably higher than the concentration determined for LAS alone.

AChE is considered a specific biomarker for organophosphate and carbamate pesticides, being commonly used to diagnose exposure of natural populations to these chemicals. However, studies published in the last decades and the present one indicate that acetylcholinesterase may also be inhibited by metals, undetermined compounds of complex mixtures of pollutants, surfactants and detergents (Olson and Christensen, 1980; Gill et al., 1990, 1991; Schmidt and Ibrahim, 1994; Labrot et al., 1996; Martínez-Tabche et al., 1996; Payne et al., 1996; Guilhermino et al., 1998). This non-

specific inhibitory effect may lead to false diagnostics and even wrong conclusions in biomonitoring studies based on the use of AChE as a specific biomarker in strongly polluted areas. Facing these results, the use of this endpoint as a specific biomarker has been questioned and a more general use for it has been suggested (Labrot et al., 1996; Guilhermino et al., 1998). From our point of view, the use of AChE as a specific biomarker should be very careful and only performed in adequate conditions. For example, when the contamination of the study area is known to be due to pesticides or when the pollutant is unknown but a suitable program of chemical monitoring is also used.

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