Available online at www.sciencedirect.com
ScienceDirect
Marine Pollution Bulletin 52 (2006) 1776–1783
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Effects of a candidate antifouling compound (medetomidine) on pheromone induced mate search in the amphipod *Corophium volutator*

Anna-Sara Krång [a,*], Mia Dahlström [b,1]

[a] *Department of Marine Ecology, Göteborg University, Kristineberg Marine Research Station, S-450 34 Fiskebäckskil, Sweden*
[b] *Department of Cell and Molecular Biology, Göteborg University, PO Box 462, S-405 30 Göteborg, Sweden*

Abstract

Environmental hazards associated with traditional, toxic antifouling coatings based on heavy metals calls for the development of alternative, environmentally acceptable antifouling compounds. Medetomidine ((±)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole) is a candidate antifouling biocide which impedes settlement of barnacles in the nanomolar range. Prior to introducing novel biocides it is of great importance to consider potential effects on non-target organisms. This study is the first to investigate the effects of medetomidine on the amphipod *Corophium volutator*, specifically effects on male mate search behaviour. In a laboratory, Y-maze bioassay, *C. volutator* males were allowed to follow female pheromones after 24 h exposure to 0 (control), 0.01 and 0.1 μg mL^{-1} medetomidine. We found that exposure to medetomidine at both concentrations significantly reduced pheromone induced mate search (by 42–71%), with fewer males crawling towards female odour. The results obtained indicate that medetomidine may impair the reproductive fitness of non-target crustaceans, an aspect that needs to be considered before further commercialisation.

Keywords: Amphipod; *Corophium volutator*; Medetomidine; Alternative antifouling compound; Pheromone; Reproductive behaviour

1. Introduction

The economic and environmental consequences of the growth of organisms on manmade structures in the sea, i.e. biofouling, are far-reaching and include increased fuel consumption, increased atmospheric waste of greenhouse gases, corrosion, loss of manoeuvrability, etc. (Champ, 2000). The need for effective antifouling coatings with little impact on the environment is urgent since traditional antifouling coatings based on heavy metals, mainly tin and copper, are persistent and highly toxic to a number of non-target organisms in the marine environment (Fent, 1996; McPherson and Chapman, 2000; Eklund and Kautsky, 2003). Organotin compounds such as tributyltin (TBT) are now banned from further use (IMO, 1999). Several alternative antifouling agents have reached the market in recent years, including the so-called organic booster biocides, such as diuron and Irgarol 1051. There is, however, a growing concern on the environmental fate and potential risks of these booster biocides and their use has been banned or restricted in many countries (Thomas et al., 2003; Konstantinou and Albanis, 2004).

Recent discoveries of candidate antifouling agents include bioactive substances of both natural (e.g. from marine sponges, octocorals or bacteria) and synthetic origin (Clare, 1996; Yamamoto et al., 1998; Dahlström et al., 2000; Burgess et al., 2003). One synthetic substance considered is medetomidine ((±)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole), classified in vertebrate pharmacology as a selective α_2-adrenoceptor agonist and an imidazoline binding site ligand (Virtanen et al., 1988;

* Corresponding author. Tel.: +46 523 18509; fax: +46 523 18502.
E-mail address: a.s.krang@kmf.gu.se (A.-S. Krång).
[1] Present address: Swedish Board of Fisheries, PO Box 423, S-401 26 Göteborg, Sweden.

0025-326X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2006.09.015

Wikberg et al., 1991). Work by Dahlström et al. (2000) shows that very low (nanomolar) concentrations of medetomidine in solution strongly inhibit the settlement of the barnacle *Balanus improvisus*, one of the major fouling organisms in Swedish waters. Medetomidine shows a high EC_{50}:LC_{50} ratio, i.e. larvae settlement was inhibited at concentrations 100,000 times lower than lethal concentration (Dahlström et al., 2000), implying low toxicity to the barnacle larvae. Medetomidine also has a strong surface affinity with implications for a minimized release of the compound to the adjacent water (Dahlström et al., 2000, 2004). These promising features have made medetomidine a potential candidate for application in antifouling coatings. Hence, the studies carried out hitherto have mainly focused on the antifouling efficacy of medetomidine and little is known about its toxicity to marine organisms.

Medetomidine is an α_2-adrenoceptor agonist and the α_2-adrenoceptor has a fundamental role in neurotransmitter release regulation and is widely distributed throughout the nervous system in vertebrates (French, 1995). Adrenergic receptors are not found in invertebrates (Roeder, 1999), but interaction with receptors with similar pharmacology as vertebrate α_2-adrenoceptor receptors has been suggested for the inhibition of the settlement of barnacle larvae (Dahlström et al., 2005). The uncertainty of the mode-of-action of medetomidine on marine fouling organisms in addition to its high potency on barnacle and tubeworm larval settlement (Dahlström et al., 2000; Dahlström and Elwing, 2006) calls for further studies on target as well as non-target organisms in the aquatic environment. Medetomidine has been found to induce pigment aggregation of melanophores in fish (Karlsson et al., 1989; Mårtensson and Andersson, 2000; Bellas et al., 2005) and it has recently been demonstrated that exposure to low (nanomolar) medetomidine concentrations decrease respiration rate in the lumpfish *Cyclopterus lumpus* (Bellas et al., 2005) and affect the burrowing behaviour of the bivalve *Abra nitida* (Bellas et al., 2006).

This study is the first to investigate the effects of medetomidine on a non-target crustacean, the amphipod *Corophium volutator* (Pallas). *C. volutator* is a tube-dwelling amphipod with great importance for the functioning of the mudflat ecosystem since it is a deposit and filter feeder (Meadows and Reid, 1966; Møller and Riisgård, 2006), as well as an important prey item for fish, larger crustaceans and migratory shorebirds (Pihl, 1985; Wilson, 1989). *C. volutator* is commonly found on shallow soft sediments in coastal areas, areas often subjected to heavy boat traffic during the spring and summer when most coastal animals have their reproductive season and, therefore, are considered most vulnerable. At reproduction, *C. volutator* males emerge from their burrows and crawl across the sediment surface in search of burrowed receptive females (Fish and Mills, 1979; Forbes et al., 1996), while females release gender-specific, waterborne pheromones that guide males (Krång and Baden, 2004). Mating can occur only for a few days following the female moult (McCurdy et al., 2000) and thus, it is essential that this search behaviour is not disturbed. We tested the hypothesis that medetomidine affects the ability of male *C. volutator* to detect and follow female sex pheromones, using bioassays with a Y-maze choice test apparatus.

2. Materials and method

2.1. Animals and sediment

Experiments were performed during May–June, 2002. Stocks of *C. volutator* were collected with 1 mm bag nets at 0.2–0.5 m depth in the Havstensfjord on the Swedish west coast (58°20′N, 11°46′E) and transported to Kristineberg Marine Research Station, Fiskebäckskil, Sweden, where the experiments took place. Individuals were sexed using the length and shape of the second antennae (Schneider et al., 1994). Females and males were acclimatized separately in 61 L aquaria ($58 \times 35 \times 30$ cm) with a 3-cm layer of sediment (see below) for at least 3 weeks with a constant supply of unfiltered surface sea water to ensure food supply. The temperature was controlled (15 °C) but the salinity had natural variations (23 ± 5 SD‰). Light regime was set to mimic natural conditions at this time of the year (16 h light: 8 h dark). *C. volutator* life history varies geographically, but in Sweden as in most other places, it has two generations per year; one over-wintering generation, which reproduces from May–June resulting in a summer breeding generation, which in turn reproduces in July–August (Möller and Rosenberg, 1982). The experiments described herein were conducted during the reproductive season of the over-wintering generation. Only adult individuals of $\geqslant 5$ mm size (measured from telson to tip of rostrum) were used, but no regard was taken of the sexual stage.

Sandy to muddy sediment (upper 3 cm) was collected from the Gullmarsfjord on the Swedish west coast (58°15′N, 11°27′E), sieved through 1 mm mesh size to remove associated macrofauna, and homogenized. Sediment dry weight/wet weight (dw/ww) ratio was 73.5% and total carbon content was $1.11\% \pm 0.09$ dw ($n = 5$) (measured with Fison Elementary analyser NA 1500 CN).

2.2. Medetomidine exposure

After acclimatization, *C. volutator* males were exposed to nominal medetomidine concentrations of 0 (control), 0.01 (50 nM) or 0.1 $\mu g\,mL^{-1}$ (500 nM) for one day (24 ± 2 h). The concentrations were chosen from a pilot test, using sub-lethal concentrations that did not cause direct observable behavioural effects, i.e. amphipods buried and no sedation or high swimming activity were observed. The concentrations are well below lethal concentrations previously described for *B. improvisus*, where some dead larvae were found at exposure to $\sim 2\ \mu g\,mL^{-1}$ medetomidine, while all larvae died at 20 $\mu g\,mL^{-1}$ (Dahlström et al., 2000). Stock solution of medetomidine (10 mM) was prepared by dissolving the hydrochloride salt of