Pharmacological agents targeted against barnacles as lead molecules in new antifouling technologies

Mia Dahlström

Department of Cell and Molecular Biology, Göteborg University, Box 462, SE-405 30 Göteborg, Sweden

ABSTRACT

The present thesis has focused on the settlement process, i.e. the attachment and metamorphosis of cypris larvae of the barnacle *Balanus improvisus* both in laboratory and field assays. It describes the use of pharmacological agents, mainly adrenoceptor active compounds, as tools for discriminating different endogenous targets in the larva, which are important for attachment and metamorphosis. Furthermore, the potential applicability of one of these imidazole-containing agents, medetomidine, as an antifouling additive against barnacles was evaluated in different field tests where antifouling activity and the release of the molecule to the ambient water were studied.

It was found that the settlement of cypris larvae was strongly inhibited (nM range) by the α_2-agonists medetomidine and clonidine in laboratory assays. Furthermore, the EC_{50}:LC_{50} ratios of both of these compounds were high (10^5-range) indicating a non-toxic mechanism of action. Both medetomidine and clonidine are classified as α_2-agonists but with known interactions also to imidazoline I_1 and I_2 binding sites in vertebrates. Thus, the ability of a range of α_2-agonists and imidazoline binding site ligands to inhibit settlement of cypris larvae was further evaluated. It was found that α-agonists and I_2 binding site ligands with settlement inhibitory effects had different pharmacological targets in cypris larvae, in spite of their similar chemical structures and their known ability to interact with both kinds of binding sites in vertebrate systems. The findings call for a more precise investigation of the physiological mechanism of these compounds and also their different endogenous targets.

In laboratory assays it was also found that cypris larvae were affected by the inherent surface chemistry of the experimental dishes. Cyprids settled to a much less extent on hydrophilic polystyrene (PS) surfaces than on hydrophobic surfaces why a further investigation was carried out to establish the importance of surface wettability. It was found that highly wettable substrates completely inhibited settlement. The findings show that *B. improvisus* larvae respond oppositely to larvae of the congeneric barnacle *B. amphitrite*, which prefer wettable substrates, and suggests that wettability might be perceived as a cue in attachment and metamorphosis.

Medetomidine but not clonidine, was found to strongly adsorb to hydrophobic and hydrophilic PS. This surface adsorption was further examined by studying effects of time, concentration, incubation media and pH on the adsorption of medetomidine, with clonidine included as a reference substance. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) measurements showed a strong medetomidine signal at the hydrophilic surface and only a weak signal at the hydrophobic surface. From these experiments it was concluded that primarily electrostatic interactions and hydrophobic interactions contribute to the interfacial accumulation of medetomidine.

Based on the strong settlement inhibition and the surface adsorption, medetomidine was predicted to show high antifouling performance in field experiments. Thus, medetomidine was included in two different paints with different paint chemistries and the release of the molecule as well as the antifouling activity were monitored over a 32-day period. Aspects of paint surface chemistry were also examined using Infrared Spectroscopy. As a comparison, tolazoline, a structural congener to medetomidine, which lacks methyl substituents, was included in the experiments. It was found that the release of medetomidine as measured with High-Performance Liquid Chromatography (HPLC) to the ambient water was not correlated to antifouling activity but that the paint with the lowest leakage rate was the most efficient against the recruitment of the barnacle *B. improvisus*. The coating with the higher leaking rate was not able to deter recruitment over the same period. The combined results allowed for a synthesis of model, which describes antifouling performance of marine coatings in terms of differential affinity states of the bioactive ingredient. The model is supported by ellipsometry measurements of surface adsorption of medetomidine and tolazoline at the liquid/solid interface of hydrophobic surfaces.

Key words: cypris larvae, settlement assay, adrenoceptor compounds, imidazoline binding site ligands, wettability, surface adsorption, ToF-SIMS, barnacle field recruitment, antifouling, release, HPLC, ellipsometry