Paramagnetic substance isolated from Parazoanthus axinelle as a new specific contrast agent

J. Strupi Suput[1], I. Sersa[2], D. Suput[1]
[1]Pathophysiology, School of Medicine, Ljubljana, Slovenia, [2]F5, IJS, Ljubljana, Slovenia

Synopsis
Pseudozoanthoxantins are fluorescent water soluble molecules isolated from Parazoanthus axinellae. One of those substances, Pax, has been shown to bind to acetylcholinesterase (AChE). Here we report that the substance is not toxic when used at 10 nM or lower concentration, and that it is paramagnetic. It decreases T2 relaxation time and may be used as a specific cotrast agent for showing AChE reach regions of brain.

Introduction
Parazoanthus axinellae is a common species inhabiting underwater caves and crevices of Mediterranean sea. So far, *Parazoanthus axinellae* was a source of a large number of yellow fluorescent water-insoluble pigments named zoanthoxantins. Similar pigments with slightly different structures were obtained from the same species and named pseudo-zoanthoxantins. No lethal or palytoxin-like activity was reported in early studies, which were primarily dealing with the structural characterization of these compounds. Their structures were thoroughly determined. They have a 1,3,5,7-Tetrazacyclopent (f) azulene (zoanthoxantin and parazoanthoxanthins) or 1,3,7,9-Tetracyclopent (e) azulene basic skeleton (pseudozoanthoxanthin, paragracine). Generally, they also differ from each other in the positions of methyl groups. In a search for new biologically active compounds from marine organisms we found that a pseudozanthoxantin isolated from *P. axinellae adriaticus* binds to electric eel acetylcholinesterase as a reversible inhibitor, similar to the action of physostigmin. Here we report some paramagnetic characteristics of the new AChE inhibitor.

Methods
The specimens of *Parazoanthus axinellae adriaticus* (Pax) were collected by SCUBA diving in the waters of island Cres (Adriatic sea, Croatia). They were frozen and transferred to the laboratory, where we kept them at -20^{o} C until use. Homogenate of the organism (250 ml) was extracted with 750 ml of absolute ethanol. A portion of the dark orange supernatant (1 ml) was passed through the Millex-GV 22 μm filter unit (Millipore, USA) and used for further purification by semi-preparative HPLC column. Active fractions were pooled again and dried as described before. Purity of rechromatographed samples was checked on the precoated F_{254} silicagel plates (Merck, FRG) using chloroform : methanol : 25% NH_4OH in a 80 : 20 : 2 ratio (v/v) as a solvent. The isolation procedure gave analytically pure compound with a molecular mass of 242 Da determined by EI-solid probe mass spectroscopy. H-NMR spectra revealed the structure of the isolated compound which is probably identical to pseudozoantoxanthin H-NMR was measured in deuterated methanol or deuterated DMSO using XL 300 MHz Varian instrument (Varian).AChE inhibitory activity was measured on purified electric eel AChE and on human erythrocyte-bound AChE, on human plasma butyrilcholinesterase (BuChE) and on the bovine cervical ganglion AChE.Inhibitory activity was expressed in inhibitory units. 1 inhibitory unit was defined as the amount of inhibitor (calculated from the dry weight of preparation) which inhibits the AChE activity by 50% at 25^{o} C. The substance was dissolved in water in 1, 10, 100 nM and 1 μM concentration. T1 and T2 decay times were measured on a 2,25 T Bruker tomograph, and data were ompared to T1 and T2 of water and control substances.

Results
Measuring the relaxation times it has been found that T1 remained nearly unchanged (10 ∀4%), while T2 decreased 40 ∀4% with 10 nM or higher concentration of Pax.Testing the AChE inhibitory activity of Pax it has been shown that it inhibits AChE reversibly in 4 uM range. Toxicity testing in rats *in vivo* revealed that the use of Pax is safe as long as the concentration of Pax in the extracellular fluid stays below 10 nM.

Discussion
As the substance is reasonably lipophilic it crosses the blood-brain barrier and may be used for identification of AChE reach regions of brain, provided that the relative concentration reaches 10 nM. Modified substances with a high affinity for AChE but even weaker AChE inhibitory activity may be developed as relatively safe contrast agents that could be used in neurobiological studies.