A MOLECULAR ANALYSIS OF MUSCARINIC RECEPTOR SUB-TYPES EXPRESSED WITHIN THE AORTA OF THE SPINY DOGFISH, SQUALUS ACANTHIAS.

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Toxicity studies on isolated aortic rings from the spiny dogfish identified a differential contractile response to cadmium, selectively inhibited (50%) by atropine, a potent inhibitor of muscarinic receptors (Evans & Weingarten, Toxicology 61:275-281, 1990; Evans et al., Toxicology 62:89-94, 1990). There had been no previous documentation of cholinergic innervation of Squalus vasculature. As the tissue specificity of receptor sub-types may reflect an underlying functional variation (e.g. adenyl cyclase inhibition or stimulation of phosphatidylinositol turnover) a greater understanding of the site of action of cadmium would be achieved by further molecular dissection of this contractile response. The aim of this work was both to establish the presence and to perform a molecular characterisation of the sub-types of muscarinic receptors in the aorta. Highly sensitive and specific methods had previously been developed to amplify muscarinic receptors using the polymerase chain reaction (PCR, and see article by JPS in this Bulletin and Bulletin 1990).

Aortic tissue (about 1g) was quickly dissected from several dogfish, and immediately flash-frozen in liquid nitrogen to prevent endogenous ribonuclease action. The tissue was polytron homogenized in lysis buffer and mRNA isolated (Fast-track mRNA extraction kit, InVitrogen, Can Diego, CA). First strand cDNA template for amplification was synthesized from about 1µg of aortic mRNA (RT-PCR kit, InVitrogen). Two rounds of muscarinic receptor amplifications were performed using degenerate primers, according to established protocols (see accompanying article by JPS in this Bulletin for details). No products were obviously amplified following the first round of PCR, as had been previously observed with Squalus cerebellum and rectal gland mRNA. However, a second round amplification using nested internal primers resulted in three clear products of the predicted length (around 800-1200bp). These were visualised by loading one-tenth of the reaction volume onto a 0.8% agarose gel containing ethidium bromide. A control reaction was simultaneously performed, excluding input cDNA, and was negative through both rounds of amplification. Future work will involve cloning these amplified products and DNA sequencing to establish them as muscarinic receptor encoding cDNAs as well as their sub-types. Full-length sequence for expression studies may be obtained by using these sequences as molecular probes to hybridize against a Squalus acanthias genomic DNA library.

In this short report we have described our initial experiments aimed at providing a more detailed molecular understanding of the target sites of action of the heavy metal cadmium. Our results suggest that muscarinic receptors are present in Squalus aortic tissue, confirming the physiological prediction. This knowledge will also facilitate the future development of more general molecular approaches to the investigation of the specific mechanisms of action of other toxic compounds in marine organisms.

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