APPARENT SECRETION OF IMMUNOREACTIVE ATRIOPEPTIN BY CULTURED CARDIOCYTES FROM THE LONGHORN SCULPIN (MYOXOCEPHALUS OCTODECIMSPINOSUS)

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Recent studies have demonstrated that both the plasma and cardiac extracts of M. octodecimspinosus contain immunoreactive atriopeptin (AP_{ir}, Evans et al., Am. J. Physiol. 257, R939-R945, 1989; Evans, Bull. MDIBL 28, 39-41, 1989). Our previous study also demonstrated that plasma levels of AP_{ir} fall significantly when this species is acclimated for one week to approximately 20% sea water (Evans et al., op. cit., 1989). As an initial step in determining the actual stimulus for this apparent reduction in cardiac secretion of AP in reduced salinities, we cultured myocytes from both atria and ventricles from M. octodecimspinosus to ascertain if the cells would secrete measurable quantities of AP_{ir}, as has recently been described for cultured cells from a freshwater chub, Gila atraria (Baranowski and Westenfelder, Life Sci. 44, 187-191, 1989).

Hearts were removed sterilely from six anesthetized fish, separated into atria and ventricles, and immediately placed in ice-cold teleost Ringer's gased with 95% O_2/5% CO_2. A suspension of myocytes was prepared by digesting a mince of atrial and ventricular tissue with 0.25% trypsin/0.2% EDTA for 60 min. Single cells were collected by pelleting and resuspended in tissue culture medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were cultured as a suspension in 30 cm^2 tissue culture flasks maintained at 20°C, in humidified atmosphere containing 3% CO_2. At days 1, 2, 3, 4, and 6, the contents of the culture flasks were poured into a centrifuge tube, centrifuged at 1000 rpm for 1 min and the supernatant aspirated and frozen at -70°C. New culture medium was then added to the cells at day 1, 2, 3, and 4. In a parallel experiment, cultured cells were incubated for 6 days before separation and freezing of the supernatant. In both experiments, the cells were saved after the final separation at 6 days and stored at -70°C for protein analysis, using the Pierce Micro BCA Protein Assay (Pierce, Rockford, Il 61105). The supernatant was thawed and extracted as previously described (Evans et al., op. cit. 1989), and refrozen at -70°C. AP_{ir} of the supernatants was determined using the radioimmunoassay developed by Epstein et al. (Bull. MDIBL 28, 16, 1989) which utilizes an antibody raised against human ANP (Amersham, Arlington Heights, IL 60005). This RIA can detect AP_{ir} at a level of 1-300 pg/sample.

TABLE 1 Rate of secretion of AP_{ir} by cultured cardiocytes

Tissue	Day 0-1	Day 1-2	Day 2-3	Day 3-4	Day 4-6	Day 6
Atrium	>500*	>250	8.3	13.8	4.7	
Atrium**		no intervening samples				>500
Ventricle	>500	>500	18	4.3	1.8	
Ventricle**		no intervening samples				>500

* Secretion rates are in pg AP_{ir}•mg protein^{-1}•day^{-1}, calculated by dividing the total AP_{ir} in the extracted culture medium by the protein content of the cells and correcting for length of culture (i.e., either 1-2 days or **six days).

It is clear from Table 1 that the cultured myocytes from both the atria and ventricles of the sculpin are capable of releasing significant quantities of AP_{ir}. In fact, the release is so substantial that the apparent AP_{ir} of the total culture medium at days 1 and

2, and after a single sample at day 6, is off-scale on the standard curve for the RIA used. The data are too preliminary for critical analysis, but suggest that culture of cardiac cells from the longhorn sculpin is possible, and that both atrial and ventricular cells are capable of secreting AP_{ir}, corroborating the earlier study of Baranowski and Westenfelder (op. cit., 1989) demonstrating that fish, but not rodent ventricles, can secrete AP_{ir}. Our future studies will more carefully examine the viability of the cells to ensure that secretion, rather than cellular lysis, can account for the release of AP_{ir} from cultured sculpin cardiocytes. Supported by NIH EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies and NSF DCB-8801572 to DHE.