ISOLATION AND IDENTIFICATION OF UNKNOWN MEDIATORS AFFECTING CARDIOVASCULAR FUNCTION

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Introduction

Cardiovascular Disease (CVD) covers a wide range of disorders and dysfunctions, and a growing global issue as the leading cause of death worldwide. Heart Failure (HF) is recognized as the most serious CVD complication, carrying a high rate of mortality. Risk of CVD and HF are correlated to several factors, but the rate of incidence is not fully explained by those risks. The underlying biochemical mechanism responsible for HF and it is probable that HF in relation to CVD may be the result of one or more unidentified proteins in the blood.

In previous work from this lab, key peptides were identified in the plasma of pigs which proved to have a strong bradycardiac effect which could lead to HF, primarily those from the protein Peptide YY (PYY). The frequency in hearts of rats were severely reduced in each case when exposed to key peptides from PYY, indicating that they have a negative inotropic effect.

Methods

Samples of blood plasma from patients were split into 3 cohorts: patients with CVD experiencing cardiac episodes (decompensated), patients with CVD after those episodes, in absence of symptoms (compensated), and healthy patients with no history of CVD. Proteins in the blood plasma from each patient were first deprotonated, then separated and isolated using a series of chromatographic techniques including size exclusion, ion exchange, and reverse-phase separation. Mass spectrometry analyses (MS and MS/MS) were then performed to screen the samples for the targeted peptides. Upon detecting the presence of the targeted peptides with MS, the identities were confirmed via MS/MS fragmentation techniques and correlated to the contributing parent proteins.

Results

Fragments of PYY2 have been observed in decompensated samples. One fragment of interest can be observed in **Figure 1A**, corresponding to m/z peak at 687 Da. In **Figure 2** the MS/MS confirmation for the fragment is displayed, identifying it as a peptide with the sequence **PEAPGEDA** unique within the neuropeptide-Y family of proteins to PYY2. The presence of sub-fragments of the parent peak indicates that the peptide sequenc is present and among other sequences identified with the same technique, providing strong evidence for the presence of PYY2, within the decompensated patient sample. It is notable that peaks at 687 Da are absent in compensated samples (**Figure 1B**).

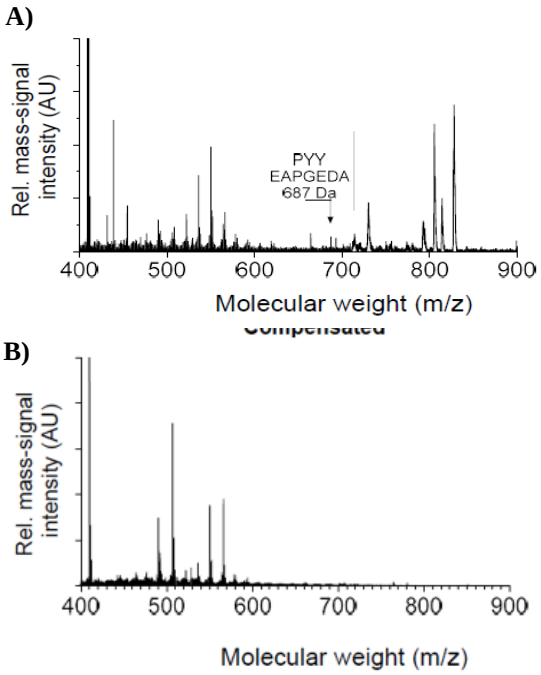

***Figure 1**: Characteristic MS mass spectrogram of isolated proteins from a **A)** decompensated and **B)** compensated CVD patient.*

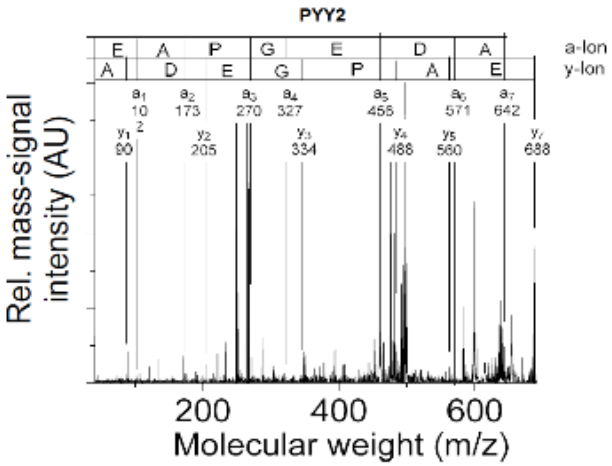

***Figure 2:** MS/MS of 687 Da mass peak found in decompensated patient.*

Discussion

The hormone PYY2 was observed to be prevalent within decompensated patients. The PYY2 sequence is largely concurrent with the peptide observed to affect heart frequency in pigs. Determining the concentration of PYY2 is an on-going task and investigation of the cardiovascular effects of the protein will be elucidated using physiological assays.