**CARDIAC TARGETING PEPTIDE; A NOVEL CARDIAC TARGETING PROTEIN TRANSDUCTION DOMAIN**

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**Background:**Cardiac diagnostics and therapeutics are limited by lack of cardiac-specific vectors. Cell penetrating peptides are small peptides able to carry intact the cell membrane barrier. Our previous work utilizing a combinatorial *in vitro* and *in vivo phage* display led to the identification of a 12-amino acid peptide, ***APWHLSSQYSRT***, which we termed***Cardiac Targeting Peptide*** ***(CTP)***, because of its ability to transduce the mouse heart tissue specifically and efficiently after an intravenous injection. We now present the result of studies undertaken to elucidate CTP’s mechanism of transduction.

**Methods and Results:**Neonatal mouse cardiomyocytes (CMCs) and mouse embryonic fibroblasts (MEFs) were incubated with 25 uM CTP dually labeled with 6-carboxyfluoroscein and Rhodamine, the latter via an ester link, susceptible to cleavage only by intracellular esterases. Hence, internalization and red fluorescence could not result from membrane attachment or fixation artifact alone. CMCs showed robust transduction within 10 minutes, with essentially no uptake by MEFs. This transduction was significantly enhanced by increasing extracellular K+ ion concentration to 20mM (p-value=0.03), with this enhancement in uptake abrogated both by calcium channel inhibitor Verapamil and Na-K+-ATPase inhibitor Digoxin. ). Increased CTP uptake secondary to cell death was ruled out by restoring extracellular K+ concentration to normal levels and resumption of CMC contractility. All transduction activity was abolished at 4°C. In a separate set of studies, mechanism of transduction was studied utilizing TRICEPs technology in a rat cardiomyoblast cell line. Co-immunoprecipitation and mass spectroscopy identified 5 potential binding partners: EPDR1, FAT2, KCNH5, MANF and WNT5a.

**Conclusions:**CTP rapidly enters CMCs in a cell specific and energy dependent manner. This transduction was enhanced by increasing extracellular K+ concentration, implying that CTP is using ion channels for cell entry. Further studies using *in vitro* cell culture are necessary to confirm the binding partner/mechanism of CMC entry by CTP.