A RHEGNYLOGIC STRATEGY FOR THE SYNTHESIS OF SIGNAL TRANSDUCTION MODULATORY, CELL PENETRATING PEPTIDES

Sarah Jones[1], Pernilla Ostlund[2], Ulo Langel[2], Matjaz Zorko[3], Iain Nicholl[1] and John Howl[1]

[1]Research Institute in Healthcare Science, University of Wolverhampton, WV1 1SB, Wolverhampton, West Midlands, UK, [2]Department of Neurochemistry, University of Stockholm, SE-106 91, Stockholm, Sweden and [3]Institute of Biochemistry of Ljubljana, SLO-1000, Ljubljana, Slovenia

Introduction

Many cell-penetrating peptides (CPP) have been utilised as biologically inert vectors.
A majority of these studies employ sychnologically organised constructs in which a bioactive cargo (*message*) is chemically conjugated to the CPP (*address*). Previously, we have adopted a sychnologic strategy to modulate intracellular signal transduction. Using chimeric constructs composed of the CPP transportan 10, conjugated to partial sequences that correspond to functional domains of signal transduction proteins, we have selectively modulated a variety of cellular activities including secretion and activation of p42/p44 mitogen-activated protein kinases [1, 2]. However, a QSAR-based algorithm can now be used to predict CPP that reside within the primary sequences of proteins [3].
We have adapted this strategy to identify CPP within signal transducing proteins including functional domains that govern protein-protein interactions.
Data presented herein indicate that it is now feasible to identify rhegnylogic sequences, containing vectoral-independent discontinuously organised pharmacophores, that are cell penetrant modulators of signal transduction pathways.

Results and Discussion

Using our rhegnylogic concept, we identified a 20 AA fragment (H-RKLTTIFPLNWKYRKALSLG-NH_2), within the first intracellular loop of the human type (a) calcitonin receptor ($hCTR_{(a)}$). This sequence, $hCTR_{(a)}^{174\text{-}193}$, includes a splice variant 16AA insert that modulates the pharmacology of hCTRs by inhibiting receptor-stimulated inositol phosphate metabolism, but facilitating the synthesis of cAMP [4] . To establish whether our rhegnylogically-organised CPP modulated hCTR pharmacology we used the human ECV304 cell line that endogenously expresses the $hCTR_{(a)}$ isoform [5]. $hCTR_{(a)}^{174\text{-}193}$ (1 µM) independently stimulated cAMP formation in ECV304 cells and augmented $hCTR_{(a)}$-stimulated cAMP by the application of salmon calcitonin (sCT) (Fig. 1. left panel). Moreover, preliminary investigations indicate that $hCTR_{(a)}^{174\text{-}193}$ directly activated heterotrimeric G proteins as measured by the initial rate of binding of [^{35}S]GTPγS to rat brain cortical membranes (Fig. 1. right panel). Confocal microscopy images captured on living cells confirmed that fluorescein-labelled $hCTR_{(a)}^{174\text{-}193}$ (1 µM) efficiently translocated the ECV304 plasma membrane.
Our rhegnylogic strategy was also applied to cytochrome C (CytC), a signalling protein integral to apoptotic events. This relatively small protein of 104 AAs was introduced into the QSAR prediction algorithm to identify putative CPP. Two rhegnylogic CPPs from the C-terminal helix of CytC, $CytC^{77\text{-}101}$ (H-GTKMIFVGIKKKEERADLIAYLKKA-NH_2) and $CytC^{86\text{-}101}$ (H-KKKEERADLIAYLKKA-NH_2) reduced the viability of U373MG astrocytoma by 39.3% and 34.9% respectively, at a peptide concentration of 30 µM.
In situ TUNEL staining confirmed that peptide-induced cell death was mediated by apoptotic mechanisms, thus eliminating necrosis and membrane perturbation commonly associated with high concentrations of CPP. Interestingly, confocal images indicated the differential sub-cellular distributions of the two peptides (Fig. 2). Rhodamine-labelled $CytC^{77\text{-}101}$ (5 µM) translocated U373MG plasma membranes to assume a perinuclear distribution (Fig. 2a), whereas $CytC^{86\text{-}101}$ (5 µM) more specifically located within the nucleus (Fig. 2b). Thus, $CytC^{86\text{-}101}$ may prove to be a useful CPP to affect the specific nuclear delivery of bioactive cargoes (peptide and nucleic acid). Future co-localisation studies will more definitively establish the intracellular ultrastructures with which these new CPPs associate. In conclusion, a rhegnylogic strategy is an effective approach to identify novel signal modulatory CPPs that influence eukaryotic cell biology.

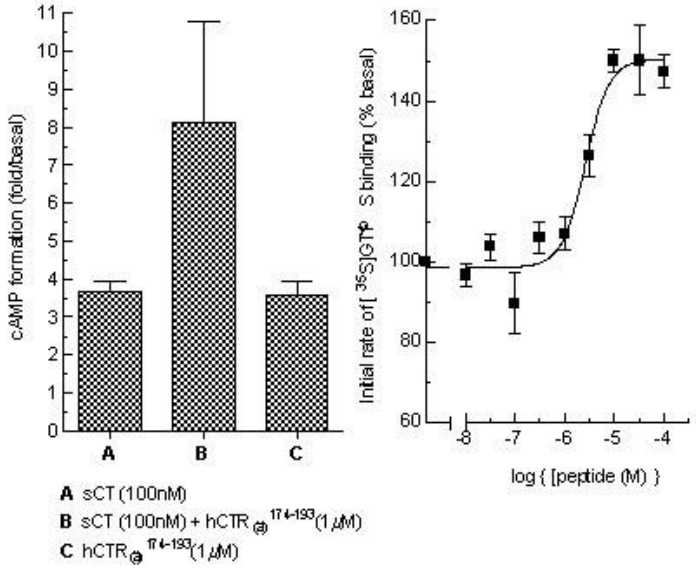

Fig. 1. Biological activities of the rhegnylogic CPP $hCTR_{(a)}^{174\text{-}193}$

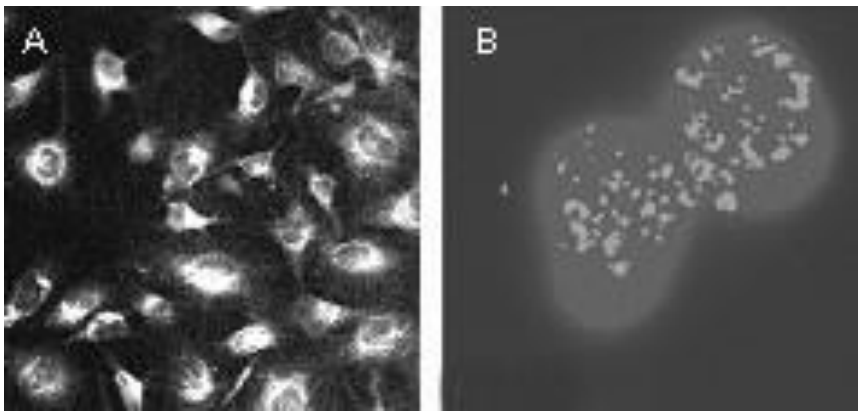

Fig. 2. Differential subcellular distribution of the rhegnylogic CPPs $CytC^{77\text{-}101}$ (A) and $CytC^{86\text{-}101}$ (B, nuclei were counterstained with DAPI, dark grey).

Acknowledgments

Keith Holding, University of Wolverhampton, U.K. provided excellent technical assistance.

References

1. Howl J., *et al*., Chembiochem., 4 (2003) 1312.
2. Jones S., *et al*,. Biochim. Biophys. Acta, 1745 (2005) 207.
3. Hällbrink M., *et al.*, Int. J. Peptide Res. Ther., 11 (2005) 249.
4. Nussenveig D.R., *et al.*, J. Biol. Chem., 269 (1994) 28123.
5. Howl J., *et al*., Endothelium, 6 (1998) 23.