Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol13Issue3abst208P.pdf

Agonist-dependent Extracellular Vesicle Release From Proximal Tubule Cells

Introduction: Originally considered a cellular waste disposal system, extracellular vesicles (ECVs) are now thought to represent a novel signalling mechanism with translational potential in disease propagation, biomarker studies and drug delivery systems. The nomenclature of ECVs is complex and represents an umbrella term unifying: exosomes, liposomes, microvesicles (MVs) and apoptotic bodies. Each subgroup is defined by their biogenesis but their formal identification largely hinges on size and the presence of differentiating surface markers. Extracellular vesicles are released into urine from all regions of the nephron[1, 2] yet the mechanisms that govern this release are poorly understood. We hypothesise that agonists known to affect intracellular vesicle traffic within proximal tubule cells would likewise affect ECV release.

Methods: Immortalised porcine proximal tubule cells (LLC-PK1) cells were grown to 70% confluency. The media was changed to a serum free environment prior to treatment with agonist and/or antagonist. Fenoldopam (1μM), 2-methylthioadenosine diphosphatetrisodium salt (MeSADP; 100μM), forskolin(10μM), and MRS 2500 tetraammonium salt (MRS; 10nM) were used. Cells were exposed to these agents for 0, 12, 24 and 48 hours. The Nanosight LM 10 instrument was used to quantify the ECV population within the media, as per our group's previously documented method[3]. Intracellular cAMP results were determined by a competitive enzyme immunoassay. Results are demonstrated as mean ± SEM and analysed using paired 2-tailed t tests.

Results: After 48 hours of stimulation**,** particles within the microvesicle size range (100-500nm) increased following treatment with fenoldopam (581.3± 103.3x10^6/ml,p <0.05 vs vehicle, 316.1 x10^6/ml) and MeSADP (563.6 ±80 x10^6/ml, p<0.05 vs. vehicle, 316.1 x10^6/ml). There was also a significant release of microvesicles after 24 hours stimulation. cAMP levels increased 4 fold following treatment with forskolina non-specific inducer of cAMPbut only 2 fold following treatment with fenoldopamyet there was no significant microvesicle release following stimulation with forskolin (282.5 ± 60.9x10^6/ml, p = ns vs. vehicle, 316.1 x10^6/ml). MeSADP stimulation of cellsin the presence of the P2Y1 antagonist, MRS, resulted in no significant increase in vesicle release (73.5 ± 15.8 x10^6/ml, p = ns vs. vehicle, 29.9 x10^6/ml). By contrast, there was no significant change in the exosome size ECV population (20-100nm) with any of these agonists.

Conclusion: Fenoldopam and MeSADP stimulation of proximal tubule cells induce release of particles within the microvesicle population size range. Our work suggests that microvesicle release is related to P2Y1 and D1 receptor stimulation but not cAMP production and further investigation into the role of ECV signalling in normal physiology is merited.

References:

1. Pisitkun, T., R.F. Shen, and M.A. Knepper (2004). Proc.Natl.Acad.Sci.U.S.A **101:** 13368-13373.

2. Alvarez, M.L., et al. (2012). Kidney Int **82**: 1024-32.

3.Oosthuyzen, W., et al. (2013). J Physiol **591**: 5833-42.