The effect of ageing and hypertension on the proton-coupled transporters PEPT1 and PEPT2 in the renal proximal tubule

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Small peptides containing 2-3 amino acids are transported by the renal proton-dependent PEPT1 (low-affinity/high capacity) and PEPT2 (high-affinity/low capacity) isoforms (Rubio-Aliaga & Daniel, 2008). Regardless of the differences in kinetics, both transporters are essential for the uptake of all possible di-and-tripeptides and are also capable of transporting a wide range of peptidomimetic drugs (Brandsch *et al.*, 2008). The reduction in renal functions associated with ageing and/or hypertension can affect reabsorption/excretion balance, a crucial process in the kidney (Mangoni and Jackson, 2004), and thus any changes in the transmembrane cotransporters PEPT1 and PEPT2 due to ageing and/or hypertension could result in imbalance. The aim of this study is to systematically investigate prospective changes of PEPT cotransporters in the kidneys with ageing and/or hypertension at the gene and protein expression, localisation and functional levels.

Kidneys were removed from different rat groups (Wistar Albino, Wistar Kyoto (WKY), and Spontaneously Hypertensive Rats (SHR)) after stunning and cervical dislocation and were snap frozen in liquid nitrogen prior to transfer to -80°C until later use. This study was approved by the Animal Ethics committee of the University of New England, and complies with the *Guide for the care and use of laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Different molecular techniques have been used to determine age-and–hypertension-related changes of PEPT cotransporters from different angles. Firstly, conventional and real-time RT-qPCR were used for relatively quantifying the gene expressions of SLC15A1 (PEPT1) and SLC15A2 (PEPT2) in two regions of the kidneys (superficial cortex and outer medulla), which are removed from different normotensive and hypertensive rat groups. Secondly, Chemiluminescent Western blot and Immunohistofluorescence staining techniques are both employed for relatively quantifying and localising the protein expression of PEPT1 and PEPT2 cotransporters. Thirdly, fluorescence spectroscopy methods were developed to measure the transport activity across the membrane of brush border (BBMV) and outer medulla (OMMV) membrane vesicles.

While we found differential expression of PEPT1 and PEPT2 cotransporters at the gene and protein levels, we also found that these proteins were no longer confined to their respective regions in the renal proximal tubule with ageing and hypertension. The changes of PEPT1 and PEPT2 profile at the gene and protein expression and localisation resulted in significant changes in the functions after examining the transport activity of the fluorophore-conjugated dipeptide β-Ala-Lys (AMCA), Glycyl-Glutamine, and Carnosine across the BBMV and OMMV. These findings suggest, for the first time, that ageing and chronic hypertension can affect localisation, expression, and function of the renal-type PEPT1 and PEPT2 cotransporters. This could have important implications on the maintenance and optimisation of nutritional amino acids and peptidomimetic drug dosage in elderly and hypertensive individuals.

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