DEVELOPMENT OF A URINE ANGIOTENSIN-CONVERTING ENZYME (ACE) ACTIVITY MEASURING TECHNIQUE

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Introduction: The expression of renal angiotensin-converting enzyme (ACE), thus the function of the local renin-angiotensin aldosterone system can be altered in hypertension and various kidney diseases. ACE is also excreted in the urine, but its activity cannot be measured due to the presence of endogenous inhibitors, so its role as a biomarker is less studied and known in cardiovascular and renal diseases.
Aim: The aim was to identify endogenous inhibitors that affect the measurement of urinary ACE activity and to develop a new measurement method that could help to clarify the role of urinary ACE as a biomarker.
Methods: ACE activity of first morning urine samples was measured by fluorescent kinetic assay after filtration (5, 10, 30 kDa pore size) or appropriate sample dilution (3-1000-fold). Ethanol precipitation and RP-HPLC separation were used for separation and identification of endogenous inhibitors.
Results: ACE activity in native urine samples from healthy individuals cannot be measured; behind this 3 endogenous, reversible ACE inhibitory compounds were isolated. By diluting urine at least 70-fold, endogenous inhibitory activity can be significantly reduced, which technique has been proved to be remarkably faster and more reproducible than conventional mechanical purification using a 10kDa pore size filter. Uric acid reduces urinary ACE activity by 25-40% (IC_{50}= 7mM) in the physiological range (1.48-4.43mM), while urea is responsible for 8-23% of endogenous inhibition (IC_{50}= 848mM) under physiological conditions (100-300mM). The third inhibitor, a non-ionic compound of less than 5kDa, is under further investigation. Under pathological conditions (>30µM), the ACE inhibitory effect of the urobilinogen also becomes pronounced (>10%).
Conclusion: Significant steps have been taken to develop a new method to measure urinary ACE activity, which can be used to routinely determine this parameter. Using our method, the role of urinary ACE activity as a potential biomarker can be better elucidated.

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