ENDOGENOUS INHIBITION OF NEPRILYSIN

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Introduction: Neprilysin is a membrane-bound zinc-dependent metalloprotease with a secreted form in the blood. Its activity has been found in human and rat tissues (predominantly in the kidneys) and in the Vero lineage cells (CCL81) which are obtained from the kidney epithelia of an African green monkey. Neprilysin plays a role in the transformation of signaling molecules, such as natriuretic peptides and angiotensin I and II. Heart failure patients have increased natriuretic peptides which is beneficial as they play a positive role in cardiovascular homeostasis, but as they are inactivated by neprilysin their supply is limited. Neprilysin inhibitors have been introduced in clinical practice to treat heart failure.
Methods: Neprilysin activity was measured using a chromogenic kinetic assay. We used human blood obtained from venous puncture. Cells of the Vero CCL81 lineage were also cultured, measurements were then carried out on the intact cells as well as the supernatant of the homogenized cells. Surprisingly, neprilysin activity (endogenous secreted enzyme) was not measurable in human blood. In line with that, the activity of exogenous recombinant neprilysin was completely inhibited by serum, suggesting the presence of a high-affinity endogenous inhibitor.
Results: There was little to no neprilysin activity in the human blood. In contrast, Vero CCL81 cells maintained the same activity in their intact state and homogenized form. The addition of human sera to Vero CCL81 cells completely inhibited neprilysin activity, suggesting that the blood contains an endogenous inhibitor suppressing circulating neprilysin. Our search for the potential inhibitors revealed the inhibitory effect of bovine and human albumin on the activity of neprilysin in blood and on recombinant neprilysin. Albumin inhibited recombinant neprilysin activity by (% of maximal inhibition here), with an affinity of (IC50 values here).
Conclusions: Our results suggest that (in contrast with the general view) neprilysin inhibiting medical drugs are not acting on the secreted (circulating) neprilysin enzyme. In contrast, circulating neprilysin activity is completely inhibited by endogenous inhibitors, such as serum albumin in humans. Our results suggest that neprilysin can only act in the tissues, where serum proteins have limited access. Endogenous neprilysin inhibition, therefore, restricts natriuretic peptide metabolism to specific tissues. These findings suggest a complex endogenous regulation of neprilysin activity and natriuretic peptide-mediated regulation of blood volume. Dysregulation of neprilysin can lead to hypertension (for which the direct cause of the disease is unknown in most cases).