Supplementary Information for

Kinetic analysis of renin and its inhibitors by detecting double-labeled peptidic substrates with an immunoassay

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Analyzing the activity of renin in plasma samples

Preparation of plasma samples: The sympathomimetic drug isoproterenol was used to stimulate renin release in mice. A single dose of 10 mg isoproterenol per kg body weight in isotonic NaCl was administered intraperitoneally to mice. One hour later, a blood sample was taken by submandibular venipuncture. Additionally, a control sample was taken from untreated mice. The blood samples were collected into hematocrit tubes with EDTA to prevent clotting. Plasma was obtained by centrifugation of the blood samples and stored at -20 °C.[1]

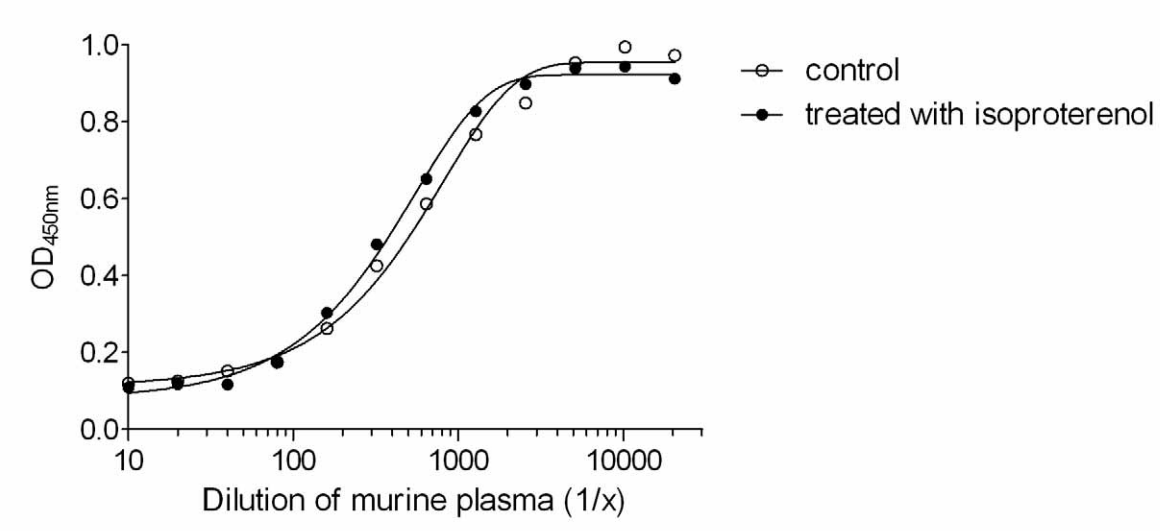
Determination of renin activity: The renin activity in the plasma samples was determined with a commercial radioimmunoassay (DiaSorin, http://www.diasorin.com). Plasma from bilaterally nephrectomized rats, which contain high levels of angiotensinogen but no renin, served as a source for angiotensinogen. This angiotensinogen was used as an excess renin substrate for the murine plasma samples, and the generation of angiotensin I was measured.[1]

Renin activity in plasma samples as determined by the radioimmunoassay:

Treated with isoproterenol: 7761 ng angiotensin I per ml per h

Control sample: 70 ng angiotensin I per ml per h

Renin assay: Peptidic substrates with the sequence 2,4-D-Aun-PEG-DRVYIHPFHLLYHN-PEG-biotin that are derived from the N-terminal part of murine angiotensinogen were synthesized and employed in the immunoassay as described in the experimental section. Both plasma samples were diluted on the microtiter plate and the degradation of the peptidic substrate is clearly detectable (Supporting Figure S1). Both samples show the same activity, although their renin content differs by a factor of 100. This can be explained by the non-specific activity of other proteases in plasma that exceed the activity of renin by far.



Supporting Figure S1. Proteolytic degradation of synthetic renin substrates in plasma.

Reference

1. Aldehni, F.; Tang, T.; Madsen, K.; Plattner, M.; Schreiber, A.; Friis, U. G.; Hammond, H. K.; Han, P. L.; Schweda, F. Stimulation of renin secretion by catecholamines is dependent on adenylyl cyclases 5 and 6. *Hypertension* **2011,** 57, 460-468.