Formation of Ang(1-7) from AngII(1-8) is largely independent of ACE2 and PRCP

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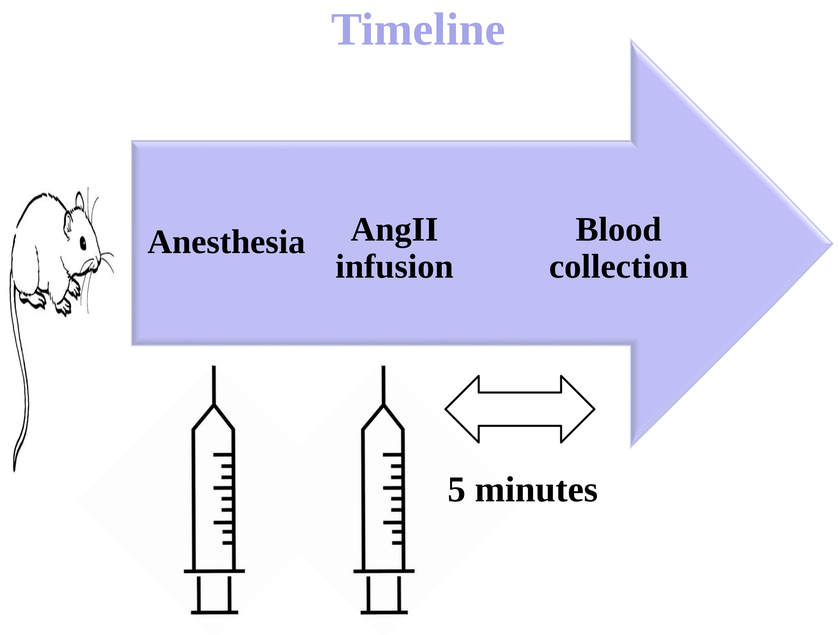
Background

- AngII(1-8) degrading mechanisms are complex including cleavage by aminopeptidases that form AngIII and carboxypeptidases like ACE2 and Prolylcarboxypeptidase (PRCP) that cleave Phenylalanine to form Ang(1-7).
- The relative importance of these peptidases to the formation of Ang(1-7) is unknown but ACE2 has been assumed to be the main Ang(1-7) forming enzyme resulting from cleavage of AngII(1-8). We tested the hypothesis that Ang(1-7) formation largely occurs independently of ACE2.

Methods

- After acute AngII(1-8) infusion to WT mice plasma concentrations of AngII(1-8), Ang(1-7) and Ang(1-5) were measured by LC-MS/MS. Additional measurements of Ang(1-7) by RIA and ELISA were performed for confirmatory purposes.
- Plasma ACE2 and PRCP activity in WT mice was measured using a fluorogenic substrate.
- ACE2$^{-/-}$ mice were crossed with PRCP$^{-/-}$ to generate an ACE2/PRCP double knockout mouse model (ACE2/PRCP$^{-/-}$).
- Male ACE2/PRCP$^{-/-}$ mice were infused with AngII and Ang(1-7) levels were measured by ELISA and compared to non-infused animals.

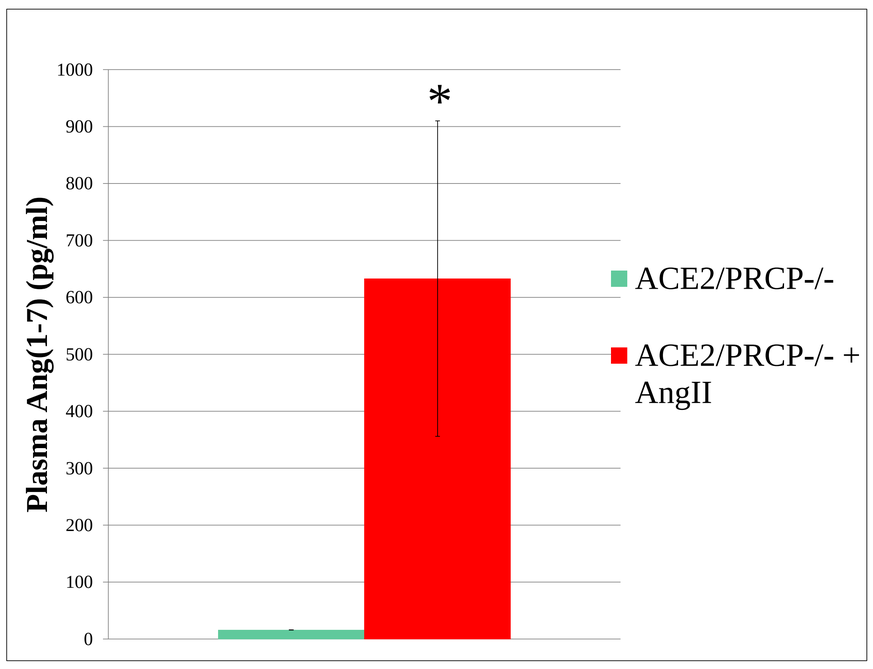


Method	AngII(1-8) (pg/ml)	Ang(1-7) (pg/ml)
MS	244 ± 21	766 ± 199
RIA	n.d.	1527 ± 240
ELISA	1012 ± 223	1137 ± 394

n.d. – not determined

Summary of results

- Following AngII(1-8) infusion to WT mice plasma Ang(1-7) levels measured by LC-MS/MS were extremely high *(Table 1)*. Similarly, high levels were also found when this peptide was measured by RIA and ELISA in AngII(1-8) infused WTs *(Table 1)*.
- In an ACE2 KO line there was no significant difference in Ang(1-7) levels as compared to WT mice and the levels of ACE2 activity in plasma of WT mice were very low (1.1 ± 0.4 RFU/µl/hr).



Conclusions

- Activity of PRCP, another Ang(1-7) forming enzyme, is extremely low at the normal plasma pH.
- Plasma Ang(1-7) levels in non-infused ACE2/PRCP$^{-/-}$ mice were not detectable by ELISA. We calculated these as 50% of the lowest detectable value (8 pg/ml) *(Figure 2)*.
- Massive formation of Ang(1-7) after AngII(1-8) infusion occurs in mice despite double ACE2/PRCP deficiency (633 ± 277 pg/ml) *(Figure 2)*.
- The exact contribution of the Ang(1-7) forming enzymes to the total degradation of AngII needs further investigation.
- Formation of Ang(1-7) during AngII(1-8) infusion is massive and largely ACE2 and PRCP-independent.
- The increase in Ang(1-7) after AngII(1-8) infusion suggests the presence of other potent Ang(1-7) forming enzyme(s).

References

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