Effects of specific inhibition of chymase on renal excretion in different rat models of hypertension

Walkowska Agnieszka, Roszkowska-Chojecka Malwina M., Gawrys Olga, Baranowska Iwona, Kompanowska-Jezierska Elżbieta

Department of Renal & Body Fluid Physiology,
M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Background

Chymase:

- a tissue angiotensin II-generating enzyme possibly engaged in the control of cardiovascular system functions;
- the ACE-independent pathway of angiotensin II synthesis is known to be overactivated in pathological conditions;
- its inhibition could have beneficial effects in the treatment of hypertension (HT)

Aim of the study

Could inhibition of chymase affect renal excretory function and body fluids composition in different rat models of hypertension?

Materials and methods

- **Acute experiments with male rats:**

1. **Groups I & II**: Unilateral-nephrectomized rats maintained on high sodium diet + chymostatin (**UNX HS+Ch,** n=5) or its solvent (**UNX HS+C,** n=8) infusion
   - Right-side nephrectomy was performed in Sprague–Dawley rats two weeks before the final acute experiments; during this time rats were maintained on a high sodium (HS, 4% Na w/w) diet.
2. **Groups III & IV**: Two-kidney, one-clip Goldblatt hypertensive rats + chymostatin (**2K1C+Ch,** n=9) or its solvent (**2K1C+C,** n=6) infusion
   - In Sprague-Dawley rats, a silver clip (0.2 mm in internal diameter) was placed on a right renal artery 28 days prior to acute experiment.
3. **Groups V & VI**: Spontaneously hypertensive rats (SHR) in the development stage (age: 7 weeks) of hypertension + chymostatin (**SHR 7+Ch**, n=8) or its solvent (**SHR 7+C**, n=9) infusion
4. **Groups VII & VIII**: Spontaneously hypertensive rats (SHR) in the established stage (age: 16 weeks) of hypertension + chymostatin (**SHR 16+Ch**, n=8) or its solvent (**SHR 16+C**, n=9) infusion.

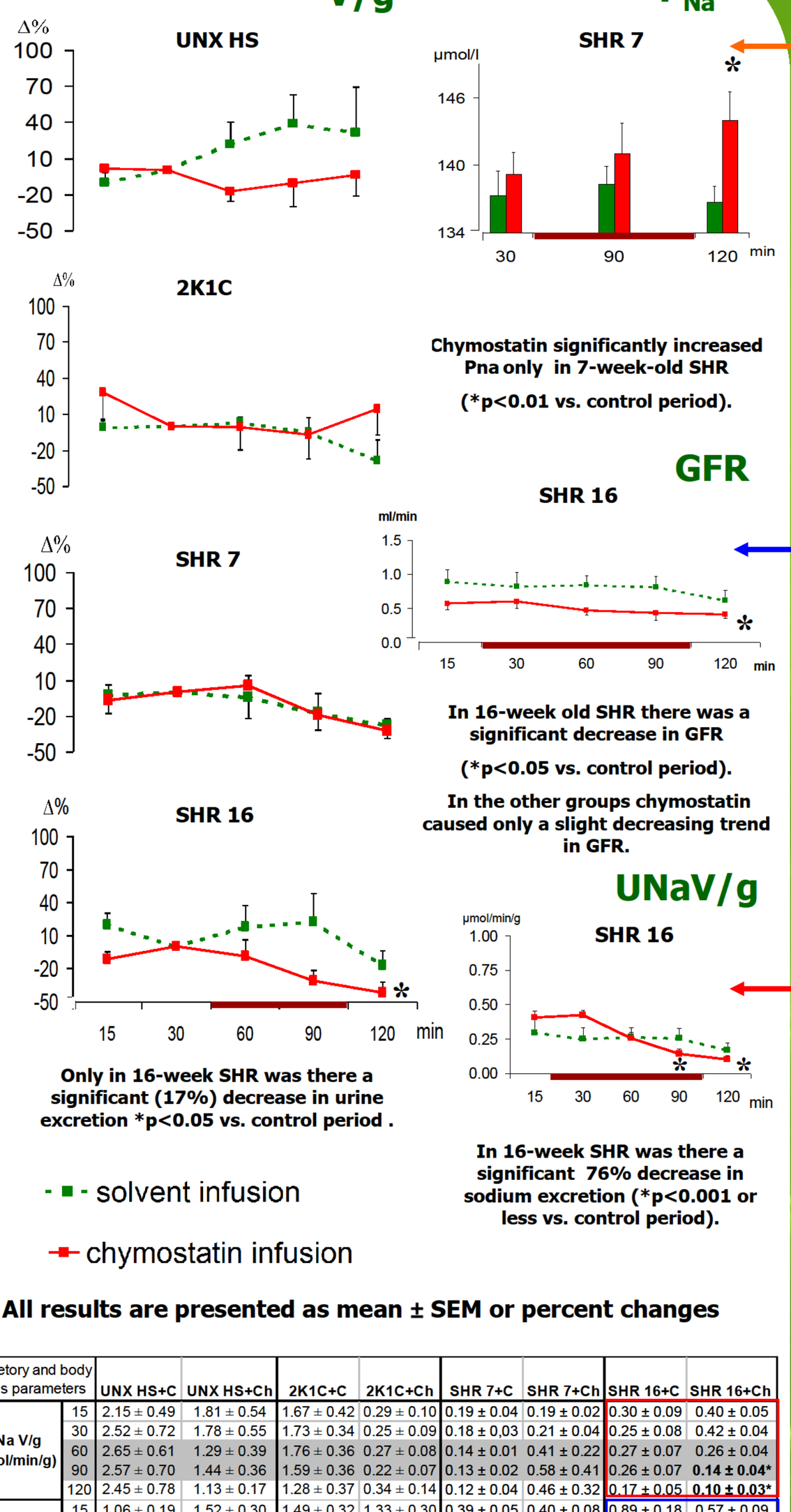
- **Anaesthesia:** sodium thiopental, 100 mg/kg BW i.p
- **Measurements:**
  - Five timed urine collections were made;
  - Blood was sampled;
  - Glomerular filtration rate (GFR, inulin clearance);
  - Plasma osmolality (Posm);
  - Plasma sodium (PNa) and potassium (PK) concentration.
- **Protocol of experiments:**
  - After control period (C), **chymostatin or its solvent** was infused i.v., followed by recovery period;
- **Excretory parameters:**
  - Urine volume was determined gravimetrically;
  - Urine (*V/g*), sodium (*UNaV/g),* potassium *(UKV/g)* and total solute *(UosmV/g)* excretion were measured and expressed per gram kidney to correct major inter-group differences in kidney size.
- **Chymostatin (Ch)/ solvent (C) dosage:**

  2 mg/kg/h infused i.v. during 1 hour (dissolved in 0.05% dimethyl sulfoxide (DMSO) with PBS and 0,9% sodium chloride - the final concentration of DMSO was 0,05%) was infused bracketed by control and recovery measurement periods.

Conclusions

- In general, most of the effects of chymase inhibition were delayed in time; some of them were even more pronounced after cessation of the drug;
- Unexpectedly, inhibition of chymase deprived GFR as well as water and sodium excretion; this was most pronounced in SHR with established HT;
- In the early stage of hypertension (7-week-old SHR) chymase blockade did not affect renal excretion but resulted in an elevation of Pna.
- Our results suggest some protective role of chymase in spontaneously hypertensive rats;
- Considering the relatively slow onset of chymostatin action, experimental protocol involving chronic administration of the drug is recommended.

Results



All results are presented as mean ± SEM or percent changes

Excretory and body fluids parameters		UNX HS+C	UNX HS+Ch	2K1C+C	2K1C+Ch	SHR 7+C	SHR 7+Ch	SHR 16+C	SHR 16+Ch
UNa V/g (μmol/min/g)	15	2.15 ± 0.49	1.81 ± 0.54	1.67 ± 0.42	0.29 ± 0.10	0.19 ± 0.04	0.19 ± 0.02	0.30 ± 0.09	0.40 ± 0.05
	30	2.52 ± 0.72	1.78 ± 0.55	1.73 ± 0.34	0.25 ± 0.09	0.18 ± 0,03	0.21 ± 0.04	0.25 ± 0.08	0.42 ± 0.04
	60	2.65 ± 0.61	1.29 ± 0.39	1.76 ± 0.36	0.27 ± 0.08	0.14 ± 0.01	0.41 ± 0.22	0.27 ± 0.07	0.26 ± 0.04
	90	2.57 ± 0.70	1.44 ± 0.36	1.59 ± 0.36	0.22 ± 0.07	0.13 ± 0.02	0.58 ± 0.41	0.26 ± 0.07	**0.14 ± 0.04***
	120	2.45 ± 0.78	1.13 ± 0.17	1.28 ± 0.37	0.34 ± 0.14	0.12 ± 0.04	0.46 ± 0.32	0.17 ± 0.05	**0.10 ± 0.03***
GFR (ml/min)	15	1.06 ± 0.19	1.52 ± 0.30	1.49 ± 0.32	1.33 ± 0.30	0.39 ± 0.05	0.40 ± 0.08	0.89 ± 0.18	0.57 ± 0.09
	30	1.06 ± 0.14	1.42 ± 0.28	1.70 ± 0.37	1.14 ± 0.29	0.46 ± 0.10	0.34 ± 0.04	0.82 ± 0.20	0.60 ± 0.09
	60	1.40 ± 0.20	1.36 ± 0.18	1.70 ± 0.27	1.27 ± 0.37	0.36 ± 0.13	0.31 ± 0.03	0.84 ± 0.14	0.47 ± 0.07
	90	1.13 ± 0.23	1.37 ± 0.18	1.60 ± 0.32	1.17 ± 0.40	0.26 ± 0.10	0.24 ± 0.03	0.81 ± 0.15	0.43 ± 0.10
	120	0.94 ± 0.23	1.01 ± 0.10	1.55 ± 0.23	1.05 ± 0.44	0.26 ± 0.09	0.25 ± 0.03	0.62 ± 0.15	**0.41 ± 0.06***
PNa (μmol/l)	30	144 ± 2	149 ± 1	143 ± 1	144 ± 1	137 ± 2	139 ± 2	138 ± 3	140 ± 2
	90	143 ± 2	149 ± 2	142 ± 1	144 ± 2	138 ± 2	141 ± 3	137 ± 3	141 ± 2
	120	144 ± 2	151 ± 1	143 ± 1	145 ± 1	137 ± 1	**144 ± 3***	137 ± 3	140 ± 2

The Table shows sodium excretion (UNaV), glomerular excretion rate (GFR) and plasma sodium concentration (Pna) in eight experimental groups.

The study was founded by the project of National Science Centre, Poland, project No. 2011/01/B/NZ4/05703