Effect of REV-ERB agonism on the endothelin axis in PER1 KO Dahl salt-sensitive rats

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Background: We have previously shown that global knockout (KO) of PERIOD-1 (PER1) in Dahl Salt-Sensitive (SS) rats results in exacerbated hypertension (HTN) and elevated levels of renal endothelin-1 mRNA (Edn1) and peptide (ET-1) following 3 weeks (wks) of a high salt (HS) diet (Zietara, et al., 2022). The recently developed compound, SR-1799, (an agonist for the nuclear receptor protein REV-ERB) from the laboratory of Dr. Thomas Burris has shown cardioprotective effects in disease models in rodents. The effects of SR-1799 treatment have not yet been characterized in SS HTN, and REV-ERB agonism could benefit rats lacking the PER1 protein.

Objective: To determine the effects of the REV-ERB agonist SR-1799 on the blood pressure (BP) phenotype and renal endothelin axis of male SS and SS PER1 KO rats given a HS diet.

Methods: Male SS and SS PER1 KO rats (8-wks-old, n = 6 per group) were maintained on a normal salt (NS; 0.4% NaCl; Dyets, Inc.) diet for 7 days and then placed on 28 days of a HS diet (4% NaCl; Dyets, Inc.). For the final 14 days of HS diet, rats were dosed with daily injections of SR-1799 (20mg/kg body weight in PBS, administered subcutaneously at 4pm). The systolic BP (SBP) of each rat was measured weekly under red light between 7-9pm using tail cuff BP measurement. Rats were sacrificed at 13 wks of age and tissue and plasma were collected for analysis. Whole kidney protein lysate and total RNA were isolated to perform ELISAs and qPCR respectively. Statistics were generated in GraphPad Prism10 using Two-Way ANOVA for protein and mRNA quantification and repeated measures mixed-effects model for SBP measurements.

Results: Following 14 days of a HS diet, there were significant increases in SBP in both SS and SS PER1 KO rats (SS NS: 156.7±7.2 mmHg, HS Wk 2: 192.1±10.2 mmHg, p=0.0009; SS PER1 KO NS: 153.3±14.8 mmHg, HS Wk 2: 192.0±18.6 mmHg, p=0.0002). Following one wk of SR-1799 treatment, SS PER1 KO rats had a significant reduction in SBP (168.0±26.0 mmHg, p=0.0137), which rose again after 2 wks of treatment (188.9±7.6 mmHg, p=0.0407). There were no significant changes in the SBP of SS controls rats from HS Wk 2 through the end of the treatment (HS+SR1799 Wk 1: 183.6±18.7 mmHg, p=0.3653; HS+SR1799 Wk 2: 178.1±24.2 mmHg, p=0.5544). Renal *Edn1* and *Ednra* mRNA levels were significantly higher in SS PER1 KO rats ($P_{genotype}$ = 0.0289 and 0.0367 respectively), with no difference in *Ednrb* mRNA levels. An interaction effect was seen in renal ET-1 peptide levels ($P_{interaction}$ = 0.0440) and no differences were seen in plasma ET-1 peptide levels.

Conclusion: Our findings suggest that daily SR-1799 injections prevented further increase in BP in SS and SS PER1 KO rats, even lowering SBP in SS PER1 KO rats after one wk of treatment; however, the effect was transient. Additionally, the rats do not see increases in

renal ET-1 peptide levels as they did after 3 wks of a HS diet in our previous studies, although there are still genotype differences in mRNA levels. Further experiments including a vehicle treated control group are necessary to fully characterize the effect of SR-1799 on SS HTN in male SS and SS PER1 KO rats.