Effects of Estrogen on Blood Pressure and Salt and Water Excretion During a Ten-day Angiotensin II Infusion Period in Ovariectomized Mice

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INTRODUCTION

Hypertension is a major risk factor for cardiovascular disease, chronic kidney disease, stroke, and other life-threatening disorders. Hypertension is where many physicians and health care providers begin when considering preventative health. Treating hypertension to reduce its prevalence often involves the regulation of the renin-angiotensin-aldosterone system (RAAS), specifically the product Angiotensin II (AngII), using drugs to inhibit angiotensin converting enzyme (ACE inhibitors) and block the angiotensin II receptor (ARBS).

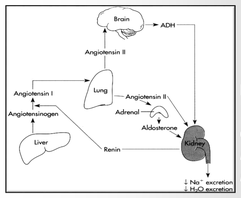

Figure 1: Basic characteristics of the RAAS which plays a major role in controlling blood pressure. *Koeppen, B. and Stanton, B: Renal Physiology, 3rd ed. Mosby, 2001, p.99*

Pre-menopausal women are known to have intrinsic protection against hypertension and cardiovascular disease compared to men of the same age. This protection is believed to be provided by estrogen. Post-menopausal women and age-matched men display a similar prevalence of hypertension-related diseases.

OBJECTIVES

- Determine if estrogen prevents or lessens AngII-induced increase in blood pressure in ovariectomized (OVX) mice
- Determine if estrogen affects body weight, food intake, water intake, and urine production in mice.

METHODS

Animals: OVX mice were obtained from Envigo (Indianapolis, IN). Ovariectomy was performed at 25 days of age. Mice were 7 weeks old at the beginning of the study. All experiments were approved by the OSU-CHS IACUC.

Procedures: The duration of the study was 16 days. Mice were divided into three groups (n=4/group): Vehicle-Placebo, AngII-Placebo, and AngII-Estrogen or AngII-E2. Mice were placed in metabolic cages (Figure 10) for a two-day acclimatization period followed by a 5-day baseline period. Surgery was conducted on day 5 followed by a recovery day, followed by a ten-day experimental (or AngII- period). Daily measurements included body weight, food and water intake, urine volume, urine osmolarity, and urine Na^+ and K^+ concentrations. A Wescor osmometer and EasyLyte analyzer were used to measure urine osmolarity and electrolyte concentrations, respectively. Mice consumed normal diet and water *ad libitum*.

RESULTS

Figure 2: Body Weight

Figure 2A: Daily Body Weight

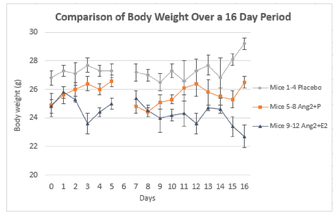

Figure 2B: Δ Body Weight

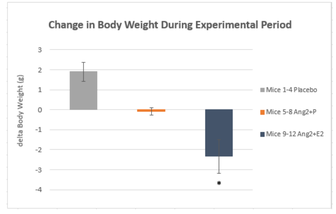

Figure 2A shows daily mean ± se body weights of each group. Surgery occurred on Day 5 and no measurement was taken on Day 6. Figure 3B shows the delta body weight of each group (i.e. difference of each mouse at end of baseline period from that at end of AngII period. * different from AngII-Placebo ($p<0.05$) and Vehicle-Placebo ($p<0.005$)

Figure 3: Food Intake

Figure 3A: Daily Food Intake

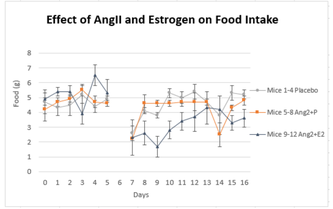

Figure 3B: Food Intake During Experimental Period

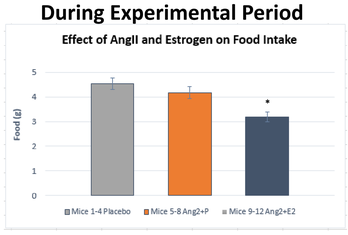

Figure 3A shows daily food intake ± se of each group. Figure 3B shows average food intake ± se of each group of each period in baseline period and AngII period. * different from respective baseline value ($p<0.001$).

Figure 4: Water Intake

Figure 4A: Daily Water Intake

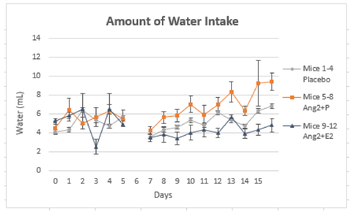

Figure 4B: Water Intake During Experimental Period

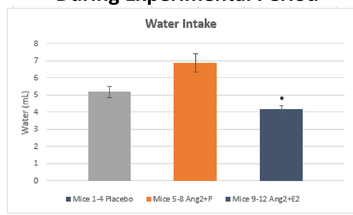

Figure 4A shows daily water intake ± se of each group. Figure 4B shows average water intake ± se of each group in the AngII period. * different from AngII Placebo ($p<0.001$). No differences occurred in the baseline period.

Figure 5: Systolic Blood Pressure (SBP)

Figure 5A: Daily SBP

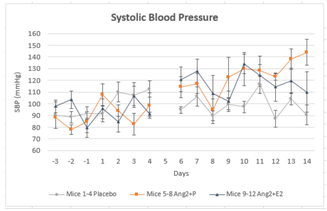

Figure 5B: SBP Baseline and Experimental

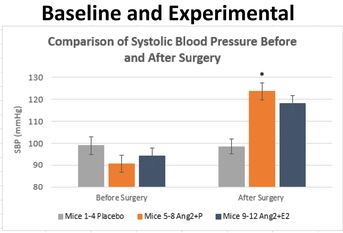

Figure 5A shows daily SBP ± se of each group. Surgery occurred on Day 5 and no measurement were taken. Figure 6B shows average SBP ± se in baseline period and AngII period. * different from Vehicle Placebo ($p<0.005$) in AngII period. No differences in baseline period.

Figure 6: Sodium Excretion

Figure 6A: Daily Sodium Excretion

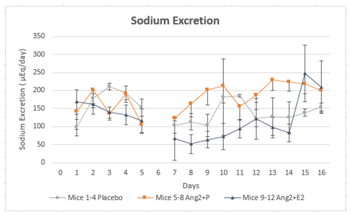

Figure 6B: Sodium Excretion During Experimental Period

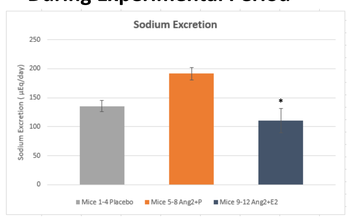

Figure 6A shows daily sodium excretion ± se of each group. Figure 6B shows average sodium excretion ± se of each group of each period in the AngII period. * different from AngII Placebo ($p<0.01$).

Figure 7: Urine Volume

Figure 7A: Daily Urine Volume

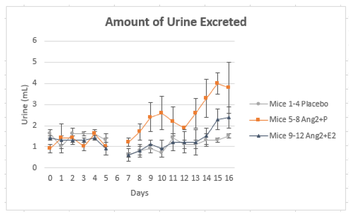

Figure 7A: Urine Volume During Experimental Period

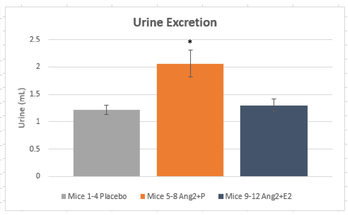

Figure 7A shows daily urine volume ± se of each group. Figure 7B shows average average urine volume ± se of each group of each period in the AngII period. * different from Vehicle Placebo and AngII-E2 ($p<0.0001$).

Figure 8: SBP Method

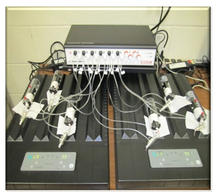

Figure 8: Kent Scientific CODA blood pressure tail cuff device.

Figure 9: Alzet Osmotic Pump

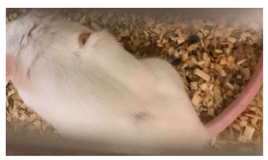

Figure 9: Subcutaneous implantation of Alzet pump which contained either Ang-II or saline. An estradiol or placebo pellet was also implanted subcutaneously next to the pump.

Figure 10: Metabolic Cage

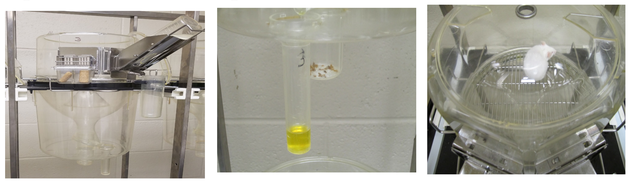

Figure 10: Metabolic cage used to measure food and water intake, along with urine excretion.

METHODS, cont'd

Blood Pressure: Systolic blood pressure (SBP) was measured daily in each mouse via the tail-cuff technique (Figure 8) using the CODA device from Kent scientific, (Torrington, CT).

Surgery: On day 5 (last day of Baseline period) surgery was performed on each mouse under isoflurane anesthesia to subcutaneously implant an Alzet minipump (Figure 9) containing either vehicle or AngII and a small pellet (either placebo or a pellet of 0.7 mg β-estradiol). The Vehicle-Placebo group contained the vehicle (i.e. saline) in the minipump and the implanted placebo pellet. The AngII-Placebo group contained AngII, which resulted in an AngII infusion rate of 1mg/kg/min, and the placebo pellet, and the AngII-E2 group had the minipump with AngII and were implanted with the E2 pellet.

Statistics: Data are shown as Mean ± SEM. Repeated measures 2-way ANOVA was used to analyze SBP (GraphPad Prism7, Ca). Fisher LSD was used for multiple comparisons. One-way ANOVA was used to measure average differences. Differences of $p < 0.05$ were considered statistically significant.

SUMMARY

1. Angiotensin II increased blood pressure in OVX mice.
2. **<u>Estrogen did not prevent AngII-induced increase in blood pressure.</u>**
3. Estrogen resulted in reduction in body weight.
4. Estrogen resulted in reduced food and water intake.
5. Estrogen reduced sodium excretion and urine volume.

CONCLUSION

Estrogen did not protect the OVX female mice from the ten-day AngII-induced increase in blood pressure. However, results suggest protection might occur in a longer time period.

Estrogen appears to affect feeding and drinking behavior and renal sodium and water excretion.

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