High salt diet increases collecting duct NO production similarly from wild type and endothelial cell ET-1 knockout mice

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The endothelin/nitric oxide (NO) axis is integral in regulating sodium transport in the renal collecting duct (CD). ET-1 acts through both autocrine and paracrine signalling mechanisms. Endothelium is one of the sources of ET-1 and this may regulate CD-derived NO production through a paracrine mechanism. We hypothesized that CD NO production is partially regulated by endothelial cell-derived ET-1 and this may be exacerbated during consumption of a high salt diet. To test this, we used the endothelial cell ET-1 KO mouse (Flox ET-1/Tie2-CRE; EC ET-1KO) and Flox ET-1 mouse (control). Mice were randomly assigned to either a week of normal salt (0.4% NaCl) diet or a week of high salt diet (4.0% NaCl). Inner medullary CDs were isolated and nitrite production (as an index of NO production) determined by HPLC. There were no significant differences in nitrite production between male and female mice of either genotype (two-way ANOVA, $p>0.05$) or diet (two-way ANOVA, $p>0.05$). For example, control male, 4% NaCl = 2713 ± 762 pmol/mg pr/ hr compared to control female, 4% NaCl diet = 3097 ± 1107 pmol/mg pr/hr (N=4-5). Nitrite production was similar between control (1332 ± 550 pmol/mg pr/hr, n= 9) and EC ET-1 KO (1511 ± 265 pmol/mg pr /hr, n = 16) mice on a normal salt diet. Likewise, there was a similar increase in CD nitrite production after being fed a high salt diet for a week (control = 2543 ± 454, EC ET1 KO = 2557 ± 317, n = 14-18). Thus, when analyzed with a two-way ANOVA, there was a significant effect of diet ($p = 0.023$) but not genotype ($p = 0.8915$, interaction $p = 0.68$). Next we tested to determine whether the increase in nitrite production seen with the high salt diet reflected an increase in the expression of NOS1 and/or NOS3. Mouse inner medullary CDs were isolated and analyzed via western blots. There were no differences in inner medullary CD NOS1 or NOS3 expression among genotype, diet, or sex. In conclusion, loss of endothelial cell ET-1 production had little effect on CD NO production. Future studies will examine the hypothesis that the autocrine actions of CD ET-1 dominate over the paracrine actions on CD NO production.