Mouse inner medullary collecting duct-3 cell NO production is regulated by ET-1, but NOS1 and NOS3 expression are not

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The inner medullary collecting ducts (CDs) regulate sodium transport through the ET-1/NO axis. Nitric oxide synthase activity, levels of ET-1, and expression of the ETB receptor are highest in the CD, making this nephron segment critical to study the regulation of the ET-1/NO axis. Previous work by our lab determined that ETB deficient rats (*sl*/*sl*) had an increase in inner medullary NOS1 expression. In agreement with this finding, it has been reported that ET-1 stimulates NOS1 derived NO production via the ETB receptor in freshly isolated rat inner medullary CDs. Interestingly, rat IMCDs express NOS1α and NOS1β splice variants, while the mouse IMCD and mIMCD-3 cells express only NOS1β. Recently, it was demonstrated that NOS1αKO mice are normotensive, while we determined that genetic deletion of CD NOS1β leads to salt sensitive hypertension. Thus, CD NOS1β is necessary for blood pressure regulation. The purpose of this study was to determine if ET-1 regulates NOS1 expression and/or activity in the mouse inner medullary CD. Given that mice express only NOS1β, this system gives us a unique opportunity to test the hypothesis that ET-1 regulates NOS1β. To test this hypothesis, mIMCD-3 cells (purchased from ATCC) were grown to confluency and given vehicle, 1, 10, 50, 100 or 500 nM ET-1 for 1 h and nitrite production determined by HPLC (as an index of NO production). There was a significant increase in nitrite production with 50 (178 ± 31 pmol/mg/hr) and 100 nM (297 ± 28 pmol/mg pr/hr) ET-1 compared to vehicle (54 ± 26 pmol/mg/hr, ANOVA with Dunnett's post hoc $P < 0.01$, $n = 5$-6). This increase was blocked by the ETB receptor antagonist, BQ788 (ANOVA, $p < 0.01$,), while the ETA receptor antagonist, BQ123, had no effect (ANOVA, $p > 0.05$). Chronic 24h treatment of ET-1 did not alter NOS1β or NOS3 expression in mIMCD-3 cells (T-Test, $p > 0.05$). As well, we blocked the endogenous ET-1 system chronically (24h) with BQ123 and BQ788, and this did not alter NOS1β or NOS3 expression (ANOVA, $p < 0.05$). Compared to the rat IMCD, there was relatively low NOS3 expression in the mIMCD-3, thus, NOS1β seems to be the predominant NOS isoform expressed in the mIMCD-3. In conclusion, blocking the endogenous ET-1 system or giving exogenous ET-1 does not alter NOS expression in the mIMCD, but it stimulates NO production and this is via the ETB receptor. NOS1β is a functionally important splice variant expressed in the IMCD and ET-1 regulation of NOS1 activity is similar between mice and rats even though mice IMCDs express only NOS1β.