ANGIOTENSIN (ANG) II STIMULATES PAPILLOGENESIS DURING LATE METANEPHRIC DEVELOPMENT.

Ali Khalili, Colleen Garrett, Renfang Song and Ihor V. Yosypiv.

Department of Pediatrics, Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, LA.

Branching morphogenesis of the ureteric bud (UB) is a key developmental process which gives rise to the ureter, pelvis, calyces and collecting ducts. We recently reported that Ang II, acting via the AT₁R receptor (AT₁R), stimulates UB branching morphogenesis during early stages of metanephric development (Yosypiv et al. Kidney International, 2008). In the present study, we tested the hypothesis that aberrant AT₁R signaling impairs papillary morphogenesis during later stages of metanephric development. We first examined the effect of genetic inactivation of subtype A of AT₁R (AT₁AR) in mice on papillary growth on postnatal (P) day P1. P1 AT₁AR⁻/⁻ metanephroi exhibited a smaller papilla compared with AT₁AR⁺/⁺ neonatal kidneys. To determine whether Ang II AT₁R directly stimulates papillary morphogenesis, we next examined the effect of the specific AT₁R antagonist, candesartan, on papillary growth using ex vivo papillary culture. Papillas were dissected from Hoxb7-GFP⁺ mouse metanephroi on P2 and grown in 3-dimentional collagen matrix gels located on air-fluid interface in the presence of media (control, n=3) or candesartan (10⁻⁶ M, n=3) for 48 hours. Images were acquired at time of dissection (“0” hours), 24 and 48 hours by time-lapse microscopy. Papillary area was determined at every time point by Slide book 4.0 image processing software and percent change in papillary area relatively to time “0” was compared between the groups. Treatment with candesartan decreased papillary area after 48 hours of culture (77±6.6 vs. 100±0%, p<0.05). In contrast, papillary area in control group at 48 hours did not differ from baseline (93±12 vs. 100±0%, p=0.6). To examine the cellular mechanisms involved in Ang II-dependent morphogenesis of papilla, we examined the effect of media (control) or Ang II (10⁻⁶ M, n=4) on BrdU incorporation in P1 mouse papillas grown ex vivo for 24 hours. Ang II increased the number of BrdU-positive cells compared with control (48±2.8 vs. 38±2.5, p<0.05). The results demonstrate that lack of AT₁AR in mice results in abnormal papillary development. Moreover, antagonism of the AT₁R directly inhibits growth of neonatal papillas grown ex vivo. Ang II induces papillary cell proliferation. We conclude that Ang II, acting via the AT₁R, stimulates papillogenesis during late metanephric development. These findings support the hypothesis that abnormal collecting system development in RAS-deficient mice is due, in part, to aberrant collecting duct growth.