Activation of renal calcium and water excretion by novel activators of the calcium-sensing receptor

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Recently, two new classes of calcium-sensing receptor (CaR) activators have been identified. The type-II calcimimetics (e.g. NPS R-467) were developed from a lead phenylalkylamine compound identified in a large-scale drug screen (Nemeth *et al.*, 1998). Type-II calcimimetics sensitise the CaR to calcium ions by binding to a site in the receptor's transmembrane region (Hauache *et al.*, 2000). Several sub-classes of L-amino acids (including aromatic, polar, and aliphatic amino acids) have been shown to act as allosteric activators of the CaR (Conigrave *et al.*, 2000). The amino acid binding site is likely to lie in the conserved N-terminal, Venus FlyTrap domain. In the kidney, the CaR is expressed in multiple sites. These include the proximal tubule, the cortical thick ascending limb (CTAL) and the medullary collecting ducts (Ward & Riccardi, 2002). Expression of the CaR in the CTAL has been linked to the control of urinary calcium excretion. Expression of the CaR in the collecting tubule, on the other hand, has been linked to the control of urinary water excretion and osmolality. In particular, CaR activation may suppress vasopressin-induced water reabsorption facilitating the excretion of solutes such as calcium, phosphate and oxalate that might otherwise contribute to the formation of renal calculi (Brown & Hebert, 1996). This pattern of expression implies roles for CaR activators in the regulation of multiple renal functions including proximal tubular transport, calcium excretion and urinary concentration. In particular, CaR-active amino acids (e.g., L-Phe and L-Ala) and type-II calcimimetics are predicted to promote calcium excretion, raise urine flow and suppress urinary osmolality.

We have examined the impact of intravenously administered L-amino acids or the type-II calcimimetic, NPS R-467 on renal calcium and water excretion. In female Wistar rats (200-300 g), anaesthetised with halothane, both jugular veins were cannulated and the animals were infused (2-4 mL/h) with isotonic physiological saline solution (140 mM NaCl, 4.0 mM KCl, 15 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$). After a 60 min equilibration, L-amino acids were infused for 60 min prior to return to the control solution. Blood samples (0.25 mL) were collected at regular intervals for analysis of creatinine, osmolality, total calcium and various amino acids. Urine samples were collected at 15 min intervals to assess flow rate, osmolality and creatinine, calcium and amino acids. In some experiments, bolus injections were administered to test for acute effects of R-467 and amino acids. The type-II calcimimetic R-467 enhanced urinary calcium excretion (~3 fold) and urinary flow rate. In addition, R-467 suppressed urinary osmolality consistent with an inhibitory action of the CaR on vasopressin-induced water reabsorption in the collecting ducts. R-467 also lowered serum total calcium levels as previously reported (Fox *et al.*, 1999). The inactive isomer, S-467 was much less effective than R-467 on all three parameters tested. Infusions of the CaR-active L-amino acid, L-Phe sufficient to raise the serum level from 0.05 mM to about 2 mM, also elevated calcium excretion (~2-fold) and urinary flow rate, and suppressed urinary osmolality. Bolus injections of L-Phe and L-Ala also acutely elevated urinary calcium excretion and flow rate and lowered osmolality.

Taken together the data are consistent with the idea that novel activators of the CaR including L-amino acids and type-II calcimimetics such as R-467 mimic the effects of elevated plasma Ca^{2+} concentration on urinary calcium excretion, flow rate and osmolality.

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