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"Prolonged Administration of Kappa Opioid Agonist Difelikefalin Retains its Efficacy on the Diuretic Response"

RATIONALE: Disease states exhibiting high amounts of edema, including congestive heart failure, hepatic cirrhosis, and hypertension, are often treated with a combination of diuretics to reduce water retention in the body. Patients treated with the loop diuretic furosemide face an increased risk for developing adverse conditions, such as hyponatremia and hypokalemia and with time loss of diuretic efficacy (diuretic resistance). In contrast, the kappa opioid agonist difelikefalin produces a sodium sparing diuresis by inhibiting hypothalamic neurons responsible for the secretion/release of antidiuretic hormone (ADH) located in the paraventricular nucleus (PVN). Consequently, inhibition of ADH release increases urine output by increasing free water clearance rather than increasing urinary sodium excretion - a common mechanism of action of other diuretics. We hypothesized prolonged administration of difelikefalin would retain its diuretic efficacy over time and avoid the development of diuretic resistance due its inhibition of ADH release. Prior studies from our lab have also shown an association between expression levels of Gα protein subunits in the paraventricular nucleus (PVN) and plasma ADH levels. Therefore, we also examined if continued administration of difelikefalin altered the levels of hypothalamic Gαq or Gαz in the PVN.

METHODS: 12 Sprague-Dawley rats were weight-matched then separated into two groups and treated twice daily for 10 days with either difelikefalin (n=6; 20 ug/kg, i.p.) or vehicle (n=6, isotonic saline, i.p.). Each day, water intake was measured, and rats were placed in metabolic cages after injection to collect 5-hr urine output. Rats were sacrificed on day 10 with harvested brains stored at -80°C. PVN tissue was collected using a 1-mm^2 punch on the cryostat. Gα subunit protein levels were determined via Western blotting. Statistical analyses of all data were performed using a 2-way ANOVA on GraphPad Prism 9.4.0 (673).

RESULTS: Our results showed that difelikefalin treatment produced a marked diuretic response that was maintained over 10 days of treatment as compared to vehicle treated rats. There were no differences in water intake, urinary sodium excretion ($U_{Na}V$), or weight between the two groups. These results highlight difelikefalin's ability to enhance free water clearance without diuretic compensation over time. Our study also found no significant difference in either PVN Gαq, Gαz, or Gαi2 subunit protein levels between treatment groups, indicating that difelikefalin potentially works via a different hypothalamic G-protein signaling pathway to inhibit ADH and produce diuresis.

SIGNIFICANCE: Hyponatremia and diuretic resistance continue to be serious issues faced by physicians when prescribing diuretics. Earlier studies from our lab have shown acute administration of the kappa opioid agonist difelikefalin produced a sodium-sparing diuresis both alone and in conjunction with the loop-diuretic furosemide. However, it was unknown if continued administration of difelikefalin would retain its efficacy or develop diuretic resistance overtime. The results from this study found no diuretic resistance or increased urinary sodium excretion after 10 days of difelikefalin administration, further supporting its clinical potential as a diuretic.