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Prolonged Administration of Kappa Opioid Agonist Difelikefalin Retains its Efficacyon the Diuretic Response

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"Prolonged Administration of the Kappa Opioid Receptor Agonist Difelikefalin Retains Diuretic Efficacy"

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Introduction

Prolonged use of the loop diuretic furosemide in the treatment of congestive heart failure is associated with potential adverse effects including hyponatremia, hypokalemia, and diuretic resistance. The kappa opioid receptor (KOR) agonist difelikefalin produces a sodium sparing diuresis by inhibiting hypothalamic neurons responsible for the secretion/release of antidiuretic hormone (ADH) in the paraventricular nucleus (PVN).

Our lab has shown that acute administration of difelikefalin produces a sodium-sparing diuresis both alone and in conjunction with the loop-diuretic furosemide.

We hypothesize that prolonged administration of difelikefalin will retain its diuretic efficacy over time and avoid the development of diuretic resistance.

Methods

Treatment Groups

- 12 male Sprague-Dawley rats were weight-matched and separated into two groups: difelikefalin (n=6; 20 ug/mL, 20 ug/kg, i.p.) or vehicle (n=6, isotonic saline, i.p.) and treated twice daily for 10 days.

Data Collection

- H2O intake and 5-hr urine output were measured daily after the first injection using metabolic cages.
- Rats were sacrificed on day 10 and harvested brains were frozen at -80°C.
- PVN tissue was collected using a 1-mm^2 punch on the cryostat.

Post-Study Analysis

- Gαq, Gαz, and Gαi2 subunit protein levels were determined in PVN tissue via Western blot.
- Statistical analyses of all data were performed using a 2-way ANOVA on GraphPad Prism 9.4.0 (673).

Results

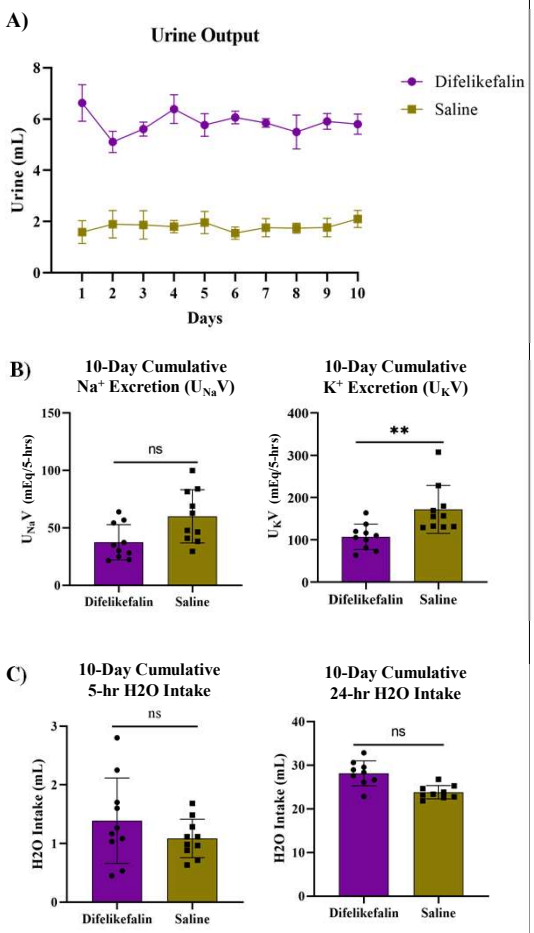

Figure 1. Effects of prolonged difelikefalin treatment on 1A) daily 5-hr urine output, 1B) 10-day cumulative urinary sodium/potassium excretion, and 1C) water intake. A) 5-hr urine was collected from rats treated twice daily with difelikefalin (n=6; 20 µg/kg, i.p) or vehicle (n=6; i.p.) for 10 days then B) analyzed for sodium ($U_{Na}V$) or potassium (U_KV) excretion. C) 5-hr and 24-hr water intake was measured between the groups.

Results

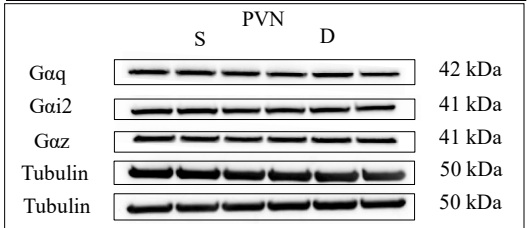

Figure 2. Gα subunit protein levels in the hypothalamic paraventricular nucleus (PVN). Rats treated twice per day with difelikefalin (D) or saline (S) for 10 days (n = 3 per group). Representative Western blots illustrating Gαq, Gαi2, or Gαz protein levels with tubulin used as a loading control.

Summary of Results

- Difelikefalin treatment produced a marked diuretic response that was maintained over 10 days of treatment as compared to vehicle treated rats.
- There were no significant differences in water intake, urinary sodium excretion (UNaV), or weight between the two groups. However, difelikefalin significantly decreased urinary potassium excretion (UKV).
- No change in the PVN Gαq, Gαz, or Gαi2 subunit protein levels was found between the difelikefalin and control groups.

Significance

These findings showed that the kappa agonist, difelikefalin, produces a marked and sustained water diuresis over 10 days. Since kappa opioids inhibit the central release of ADH, it is likely that the combined administration of difelikefalin and a loop diuretic (furosemide) may offer a novel approach to treat edematous patients without causing diuretic resistance or excessive loss of sodium or potassium.

Acknowledgments

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