The Kappa Opioid Agonist Difelikefalin Reverses Diuretic Resistance to Furosemide

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Introduction
Furosemide (Fur) is a loop diuretic used to remove edema in heart failure patients. It acts to block the Na+/K+/2Cl- cotransporter at the loop of Henle in kidney nephrons, resulting in natriuresis and diuresis. Prolonged administration of furosemide has been shown to have reduced efficacy leading to decreased total urine output, known as diuretic resistance. We hypothesize that an increase in the secretion of antidiuretic hormone (ADH) contributes to diuretic resistance after repeated furosemide administration, likely due to an increased reabsorption of water by the kidneys.

Aims of the Study
It is known that kappa opioid agonists act in the paraventricular nucleus (PVN) of the hypothalamus to inhibit ADH synthesis and secretion. Hence, we performed studies to determine whether combination therapy of furosemide plus a kappa opioid agonist, difelikefalin (Dif), can reverse diuretic resistance.

Methods

Drugs were administered via intraperitoneal injection daily. After treatment, rats were placed in metabolic cages and urine was collected over 5-hours. Rats had access to water except for the last 10 days. After thirty days, rats were sacrificed, and brains were collected. Punches of the paraventricular nucleus (PVN) were used for Western analysis of Gαq and Gαz levels with tubulin used as a loading control.

Results

Values for urine output are shown in Figure 1. No statistically significant difference was observed in urine output between treatment groups as well as no difference in PVN Gαq levels between rats treated with furosemide or combination therapy. Tubulin was used as a loading control. Group 1 (n=3) and Group 2 (n=3) levels are shown.

Findings and Conclusion
The findings of these investigations are summarized below.

- Groups 1 and 2 showed a decrease in urine output after prolonged furosemide administration thus demonstrating diuretic resistance (Fig. 1).
- Combination treatment of rats with furosemide plus difelikefalin (Group 2) produced significantly increased daily 5-hour urine output as compared to rats treated only with furosemide (Group 1). The difference in magnitude of urine output between groups was greater after water was taken away (Fig. 1).
- Rats treated only with furosemide showed a significant increase in 5-hour water intake as compared to rats that received combination treatment (Fig. 2).
- Western blot showed no difference in PVN Gαq levels between treatment groups as well as no difference in PVN Gαz levels between treatment groups (Fig. 3).
- These findings demonstrate that difelikefalin reversed the impaired diuretic response to chronic furosemide in rats independent of changes in PVN Gαq and Gαz levels. Since kappa opioid agonist ADH secretion, it is possible that combination diuretic therapy with furosemide and difelikefalin may offer a new approach to treat diuretic resistance to loop diuretics. The data support our hypothesis that an increase in ADH contributes to diuretic resistance after repeated furosemide administration.