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**“The Kappa Opioid Agonist Difelikefalin Reverses Diuretic Resistance to Furosemide”**

**BACKGROUND:** Furosemide is a loop diuretic used to remove edema in heart failure patients. Prolonged administration of furosemide has been shown to have reduced efficacy and decreased total urine output, known as diuretic resistance. We hypothesize that an increase in the secretion/release of antidiuretic hormone (ADH) contributes to diuretic resistance after repeated furosemide administration, likely due to an increased reabsorption of water by the kidneys. It is known that kappa opioid agonists act in the hypothalamus to inhibit ADH synthesis and secretion. Therefore, we performed studies to determine whether combination therapy of furosemide plus the kappa opioid agonist, difelikefalin, can reverse diuretic resistance. Our lab has previously shown an association between increased paraventricular nucleus (PVN)  $G\alpha q$  protein levels and increased ADH during water retention. Therefore, we examined whether difelikefalin increases urine output by altering PVN  $G\alpha q$  protein levels in rats co-treated with furosemide.

**METHODS:** Sprague-Dawley rats were treated daily with furosemide (10 mg/kg, i.p.) for 10 days and then either continued (days 11-30) on furosemide (Group 1, n=6) or treated with furosemide plus difelikefalin (Group 2: 20  $\mu$ g/kg, i.p., n=6). Following drug injection, rats were placed in metabolic cages with access to water except for the last 10 days and urine was collected over 5-hours. At the end of the study, rats were sacrificed, and brains were collected. Punches of the paraventricular nucleus (PVN) were used for Western analysis of  $G\alpha q$  levels.

Day of TX	5-Hour Urine Output (ml)		5-Hour Water Intake (ml)	
	Group 1	Group 2	Group 1	Group 2
	TX: Saline		Saline	
Control	1.8 ± 0.2	1.8 ± 0.4	1.5 ± 0.6	1.1 ± 0.2
	TX: Fur		Fur	
1 (peak)	11.3 ± 0.6	11.5 ± 0.4	3.0 ± 0.5	1.9 ± 0.4
10	8.3 ± 0.5*	7.3 ± 0.4*	5.8 ± 0.5*	2.9 ± 0.7 $\tau$
	TX: Fur		Fur + Dif	
11	8.6 ± 0.2*	10.6 ± 0.3 $\tau$	5.9 ± 0.5*	1.8 ± 0.2 $\tau$
20	8.1 ± 0.3*	9.4 ± 0.5* $\tau$	5.0 ± 0.8*	1.2 ± 0.1 $\tau$
	TX: Fur		Fur + Dif	
21	6.4 ± 0.2*	11.1 ± 0.5 $\tau$	---	---
30	7.7 ± 0.5*	11.3 ± 0.8 $\tau$	---	---

\* , sig different (p<0.5) than respective group Day 1 (peak FUR value);  $\tau$ , sig different (p<0.05) than respective Fur+DIF value on same day.

**RESULTS:** Table: Furosemide (Fur) markedly increased urine output in rats (Groups 1 and 2; day 1, peak). Compared to day 1, the diuresis to daily furosemide was significantly decreased over subsequent days whether rats had access to drinking water (days 11-20) or not (days 21-30). In contrast, combination treatment with difelikefalin and furosemide restored the diuresis to day 1 levels in rats regardless of water access (Group 2). Interestingly, difelikefalin also prevented the furosemide-induced increase in drinking. Western blot analysis showed no difference in PVN  $G\alpha q$  levels between treatment groups.

**CONCLUSION:** These findings demonstrate that difelikefalin reversed the impaired diuretic response to chronic furosemide in rats independent of changes in PVN  $G\alpha q$  levels. Since kappa opioids inhibit ADH secretion, it is possible that combination diuretic therapy with difelikefalin and furosemide may offer a new approach to treat diuretic resistance to loop diuretics.