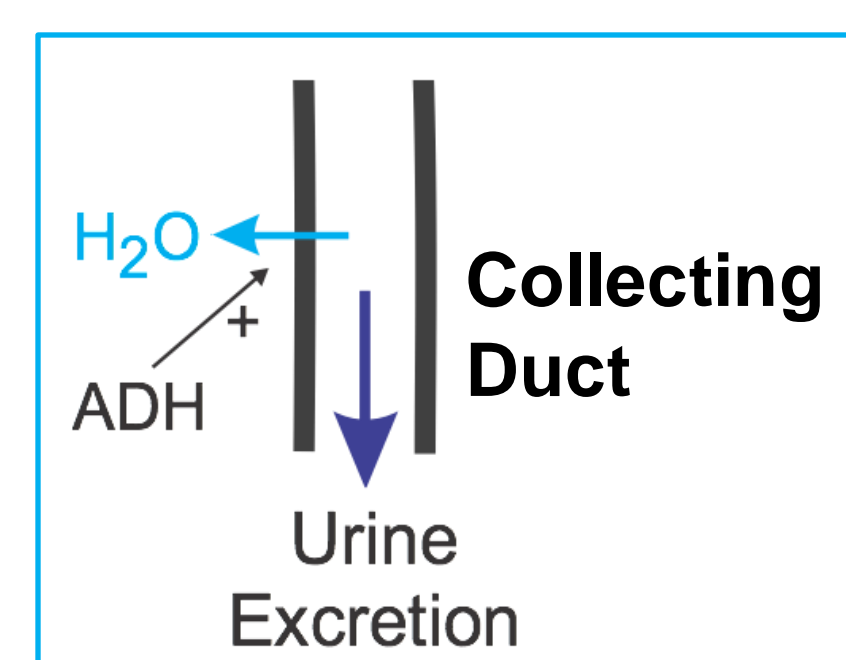
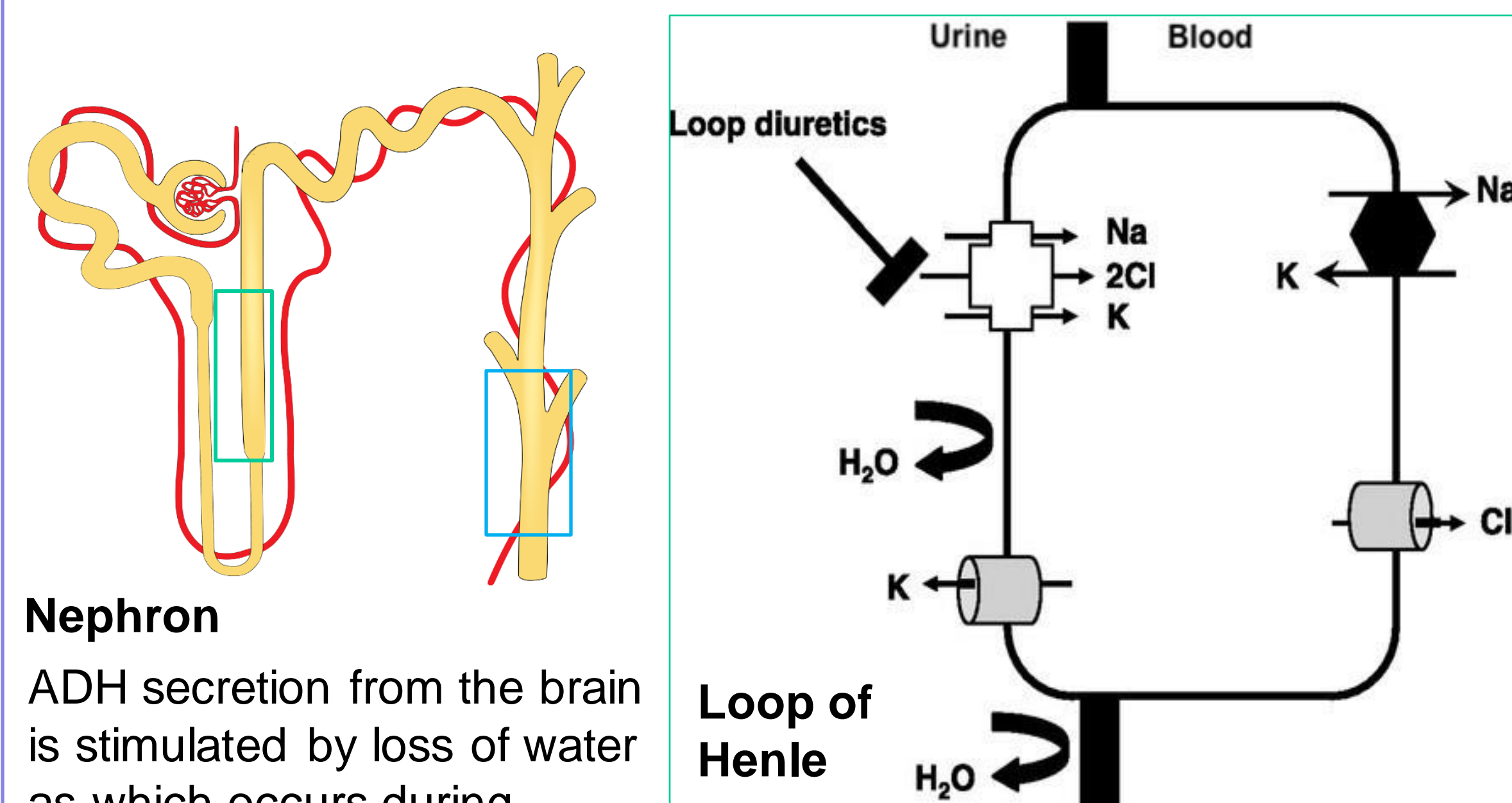


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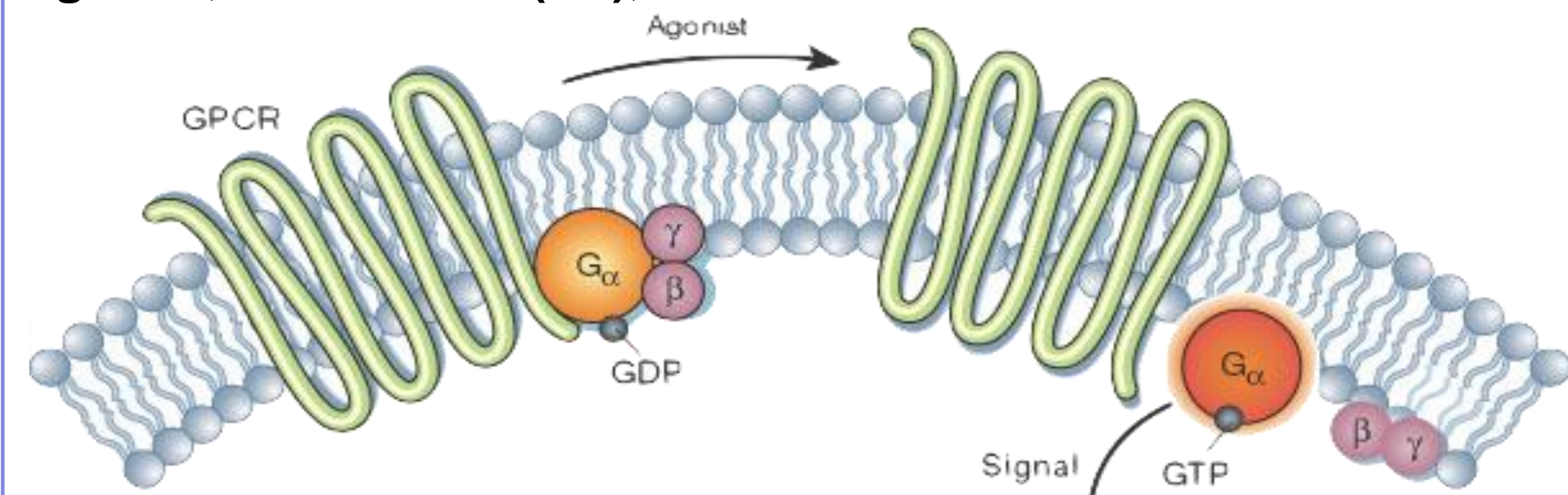
Introduction

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.

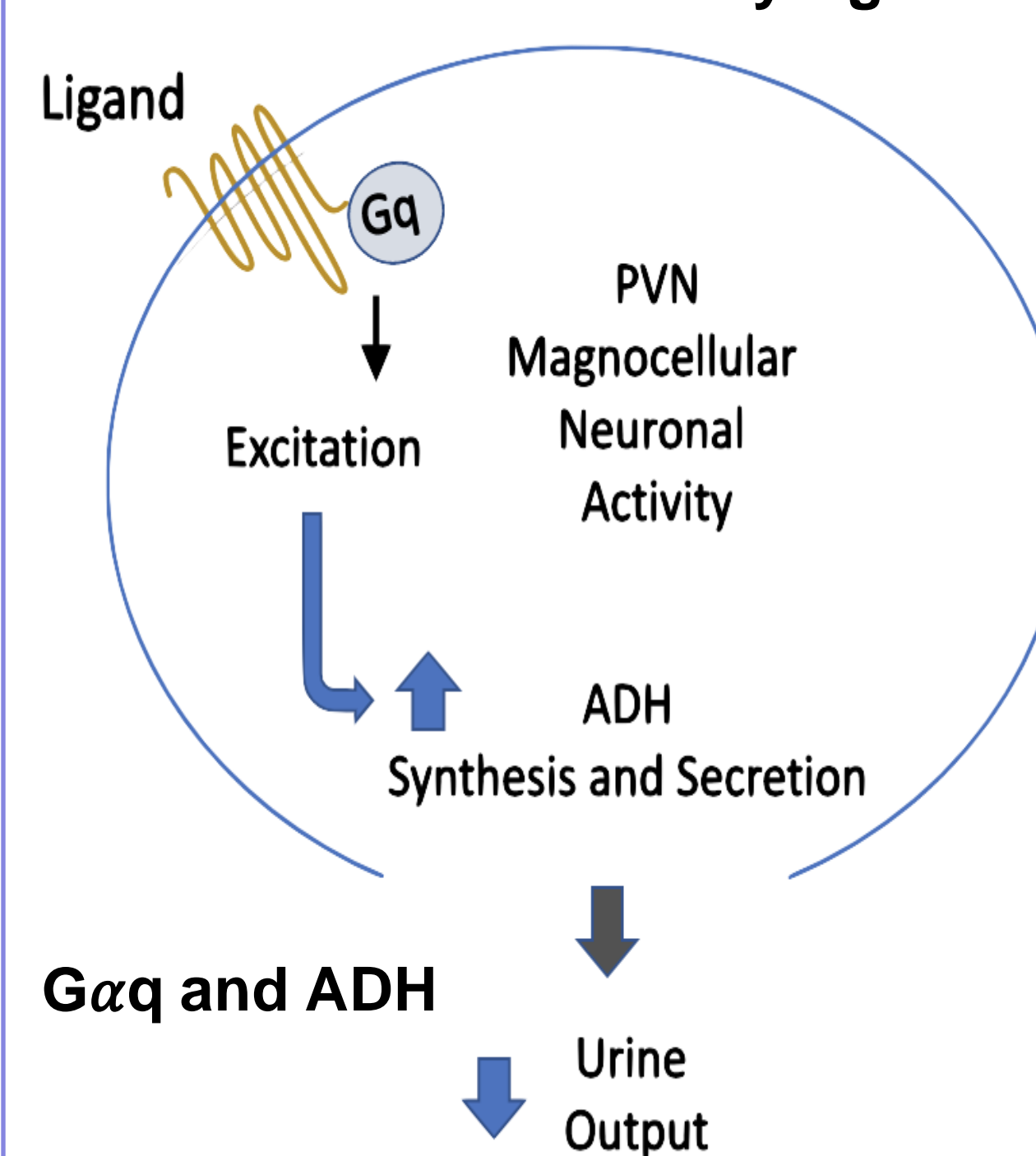


Aims of the Study

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.**

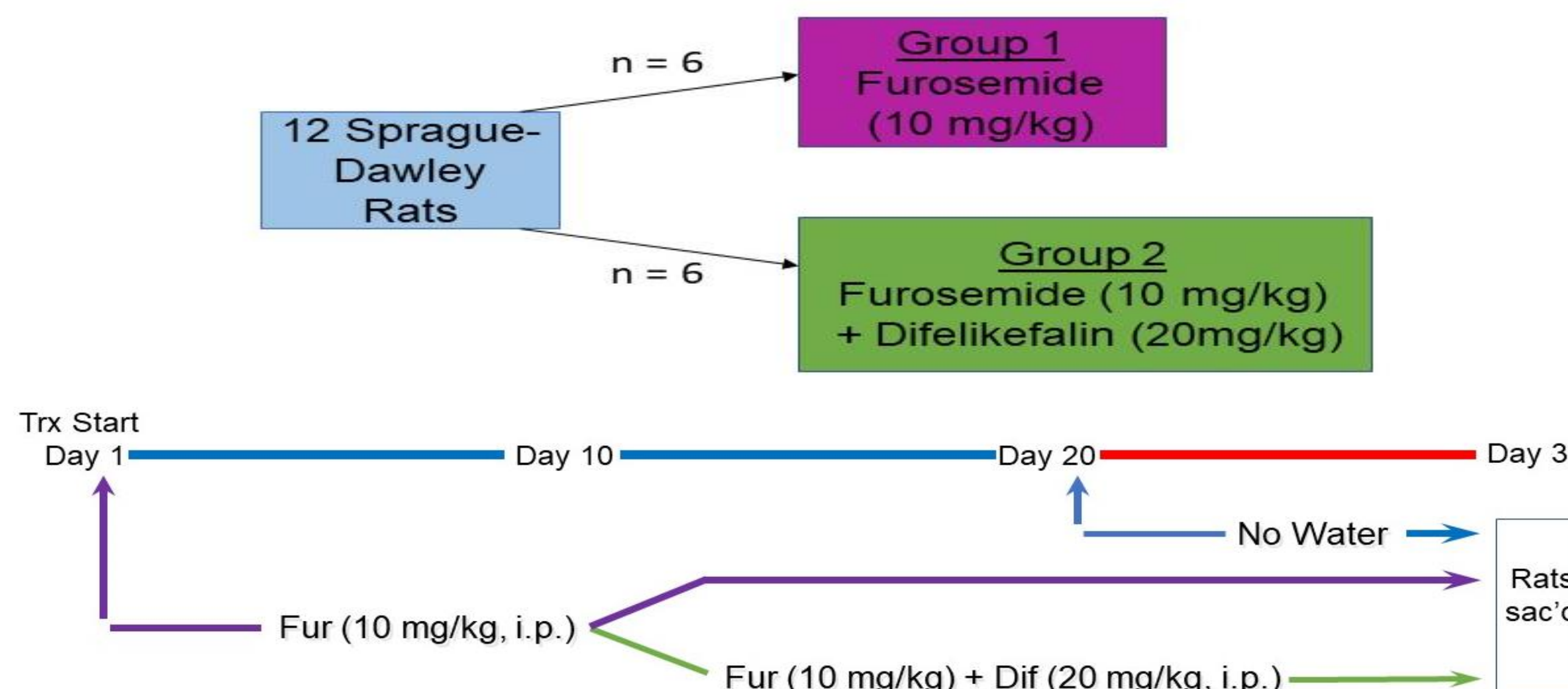


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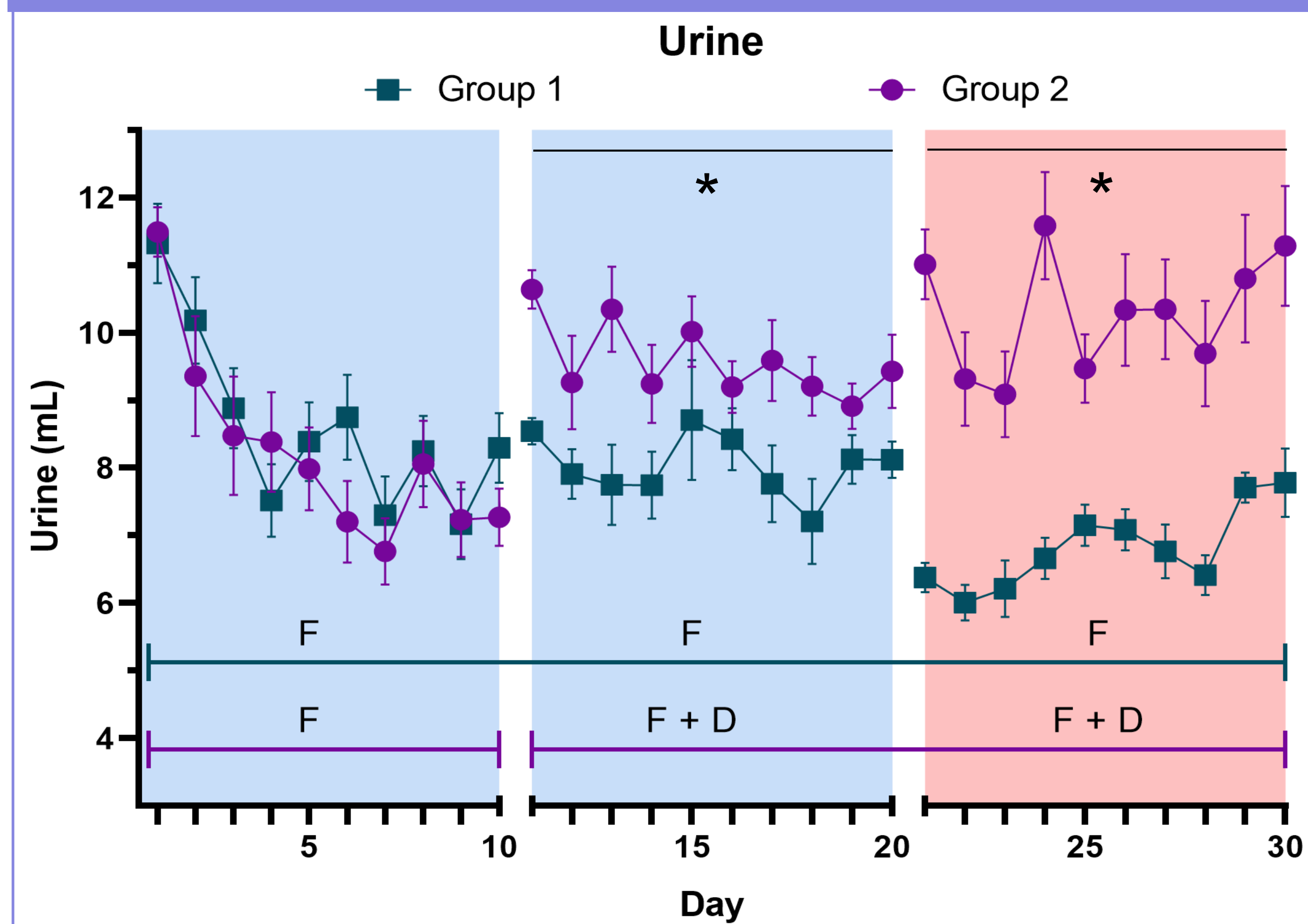
Our lab has previously shown an association between increased PVN Gαq protein levels and increased plasma ADH during water retention. Therefore, we also **examined whether difelikefalin increases urine output by altering PVN Gαq protein levels in rats co-treated with furosemide.** Gαz levels were also examined to support previous studies done by our lab.

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



	Access to Water
	No Water
F	Furosemide
F + D	Furosemide + Difelikefalin

Figure 1. Daily 5-Hour Urine Output in Response to Administration of Furosemide or Combination Therapy. Values are mean ± SEM for urine output for Groups 1 and 2 over thirty days. Both groups received furosemide for ten days. Group 1 received furosemide for the following twenty days. Group 2 was given furosemide and difelikefalin for the last twenty days. Access to water was denied for the final ten days. * P<0.05, combination treatment (Group 2) significantly different than respective furosemide treatment (Group 1) over the same 10 days. Baseline mean ± SEM 5-hour urine output for Group 1 was 1.9 ± 0.2 and for Group 2 was 2.2 ± 0.4.

Results

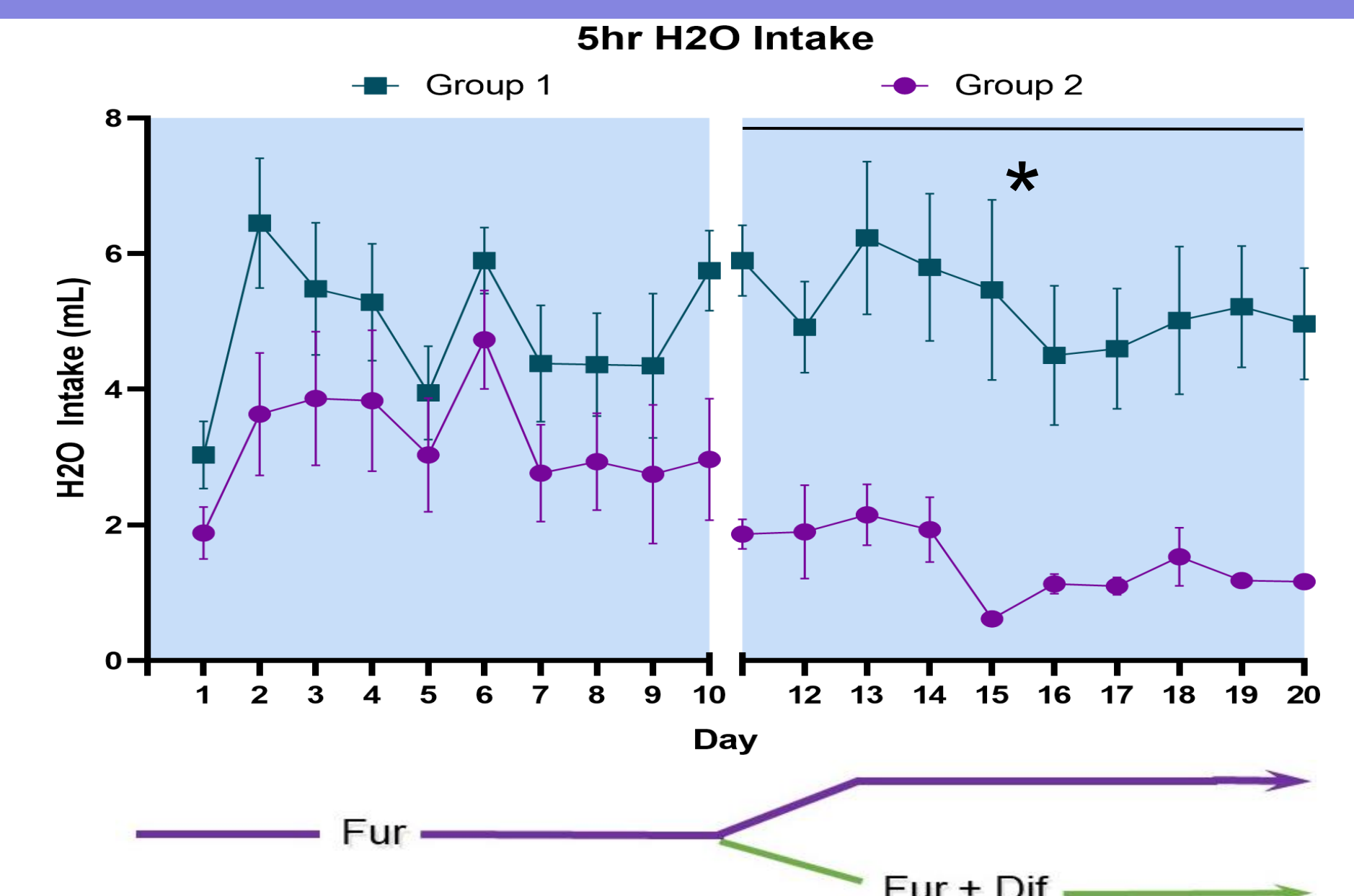


Figure 2. 5-Hour Water Intake. Values are mean ± SEM. Depiction of water intake over a 5-hour period in response to furosemide (Group 1) and combination therapy (Group 2) with access to water. * P<0.05, combination treatment (Group 2) significantly different than respective furosemide treatment (Group 1) over 10 days.

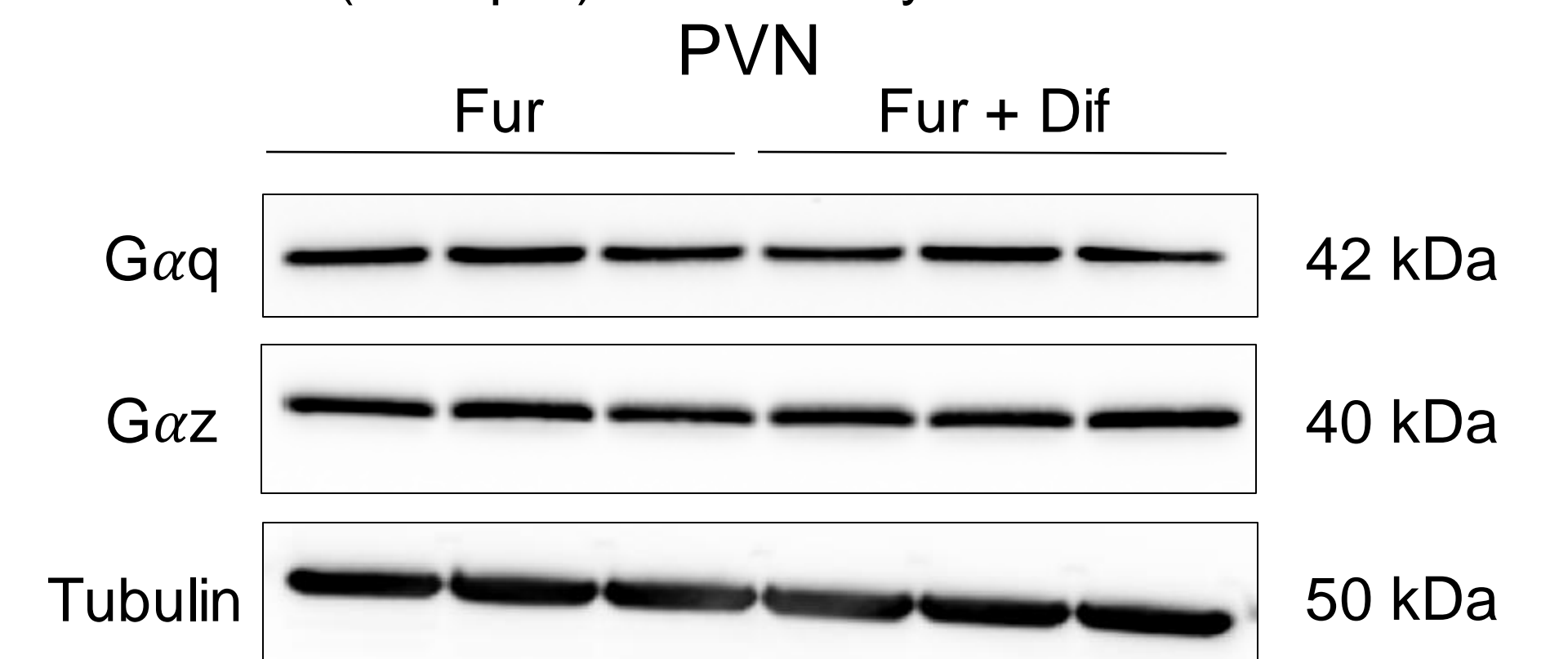


Figure 3. Gα subunit protein levels in the hypothalamic paraventricular nucleus (PVN). Western blot analysis of Gαq and Gαz levels in hypothalamic PVN of rats treated with furosemide or combination therapy. Tubulin was used as a loading control. Group 1 (n=3) and Group 2 (n=3) levels are shown.

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 opioids inhibit 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 supports our hypothesis that an increase in ADH contributes to diuretic resistance after repeated furosemide administration.