The Role of Hindbrain GABA-ergic Signaling in the Modulation of Anorexia and Malaise

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Introduction

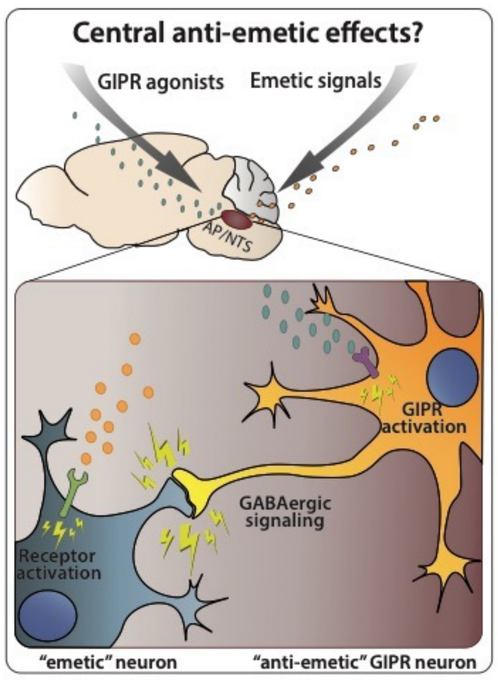

Fig.1: Working model: *GIPR activation may counteract GLP-1/chemotherapy–induced malaise via direct modulation of the AP/NTS circuitry. Given the inhibitory nature of the GIPR-expressing neurons, one can speculate the existence of a local inhibitory network within the caudal hindbrain that could be exploited via GIPR activation to reduce hindbrain GLP-1R–mediated emesis and nausea.*

Nausea and vomiting are two of the most distressing side effects in treatments for diseases such as diabetes, obesity, and cancer which often leads to **poor quality of life** and **treatment discontinuation**.

Emesis and nausea are largely controlled by the central nervous system (CNS), specifically by the **area postrema (AP)** and the **nucleus of the solitary tract (NTS**), two adjacent hindbrain nuclei.

Recent work from our lab has shown that glucose-dependent insulinotropic polypeptide receptor (**GIPR**) agonism reduces the occurrence of nausea and emesis induced by Glucagon-like peptide 1 **(GLP-1) based therapeutics**.

A high percentage of the GIPR expressing neurons co-express the **inhibitory neurotransmitter GABA** (*Gad2*) in the AP/NTS, suggesting that GIPR agonism may exert its anti-emetic effects by indirectly inhibiting emetic AP/NTS neurons, through increasing local GABA release.

Methods and Aims

By performing AP/NTS-targeted injections of adeno-associated viruses (AAVs) which cre-dependently encode for excitatory (G_q-coupled) designer receptors exclusively activated by designer drugs (DREADDS) in the Gad-cre rats, this study aims at:

1. **Determine whether chemogenetic activation of Gad$^+$ neurons in the AP/NTS attenuates Semaglutide-induced malaise in rats.**
2. **Test if chemogenetic activation of Gad$^+$ neurons in the AP/NTS attenuates malaise induced by the chemotherapeutic agent cisplatin in rats.**
3. **Evaluate the effects of chemogenetic activation of Gad$^+$ neurons in the AP/NTS on gastric emptying in rats.**
4. **Quantify in vivo co-localization of cFos expression induced by the chemotherapeutic agent cisplatin and chemogenetic activation of Gad$^+$ neurons in the AP/NTS in rats.**

Results

Activation of AP/NTS GABA-ergic neurons enhances semaglutide-induced hypophagia while reducing malaise

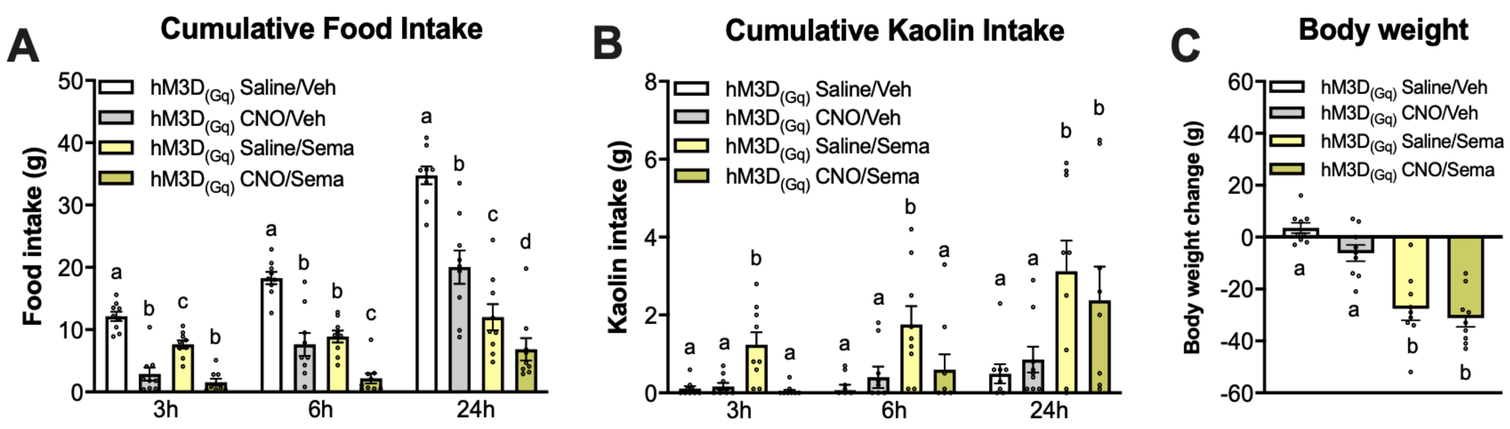

Fig.1: (A) *CNO-induced (1mg/kg IP) activation of AP/NTS GABA-ergic neurons alone induces anorexia. When CNO is combined with semaglutide (5nmol/kg IP), the treatment results in a more profound anorexia in rats than semaglutide alone. (B) Compared to semaglutide administration, the combined treatment reduced pica behavior (the consumption of non-nutritive kaolin clay, a well validated a proxy for nausea in animals that, like rodents, lack the emetic reflex).* ***(C)*** *Both combined treatment and semaglutide treatment cause reduction in body weight 24h post injection. The suppression of food intake and reduced pica suggests that GABA-ergic signaling within the AP/NTS can inhibit emesis/nausea without reducing the ability of semaglutide to reduce feeding. All Data analyzed with repeated measurements 2-way ANOVAs followed by Tukey post hoc test. Data expressed as mean ± SEM. Means with different letters are significantly different from each other (P < 0.05).*

Enhancing AP/NTS GABA-ergic signaling reduces malaise induced by cisplatin while enhancing hypophagia

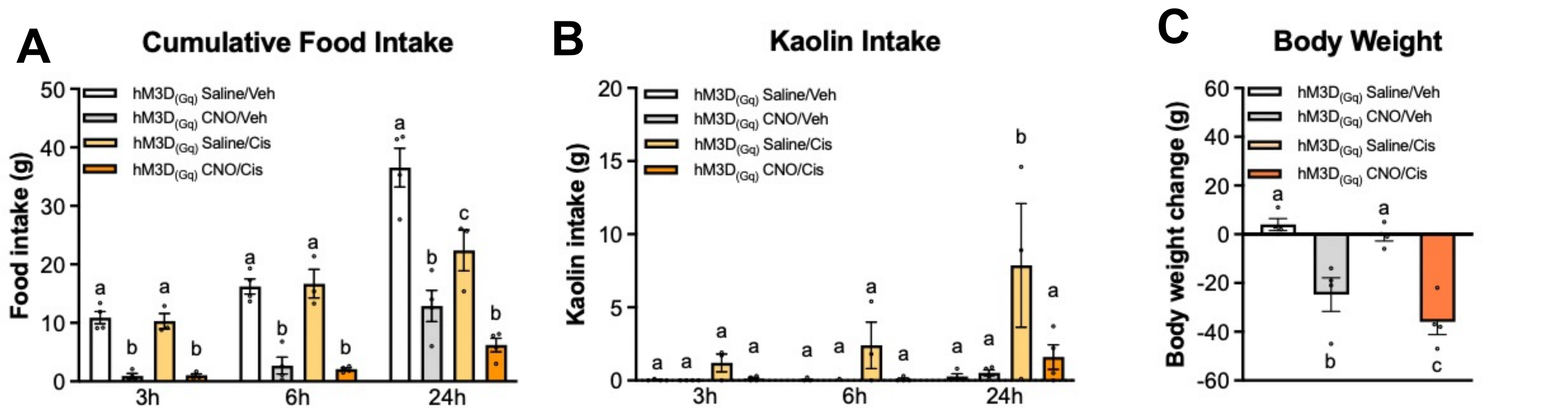

Fig.2: (A) *Both cisplatin (6 mg/kg IP) and CNO (1mg/kg IP) induced anorexia.* ***(B)*** *Remarkably, CNO treatment reduced pica behavior induced by cisplatin.* ***(C)*** *Activation of GABA-ergic neuron caused further weight loss when combined with cisplatin. The reduced pica suggests that inhibitory GABA signaling within the AP/NTS can inhibit emesis/nausea induced by chemotherapeutic agent cisplatin but it also aggravates the anorectic effects of cisplatin . All Data analyzed with repeated measurements 2-way ANOVAs followed by Tukey post hoc test. Data expressed as mean ± SEM. Means with different letters are significantly different from each other (P < 0.05).*

Validation of chemogenetic tools for selective activation of AP/NTS GABA-ergic neurons and evaluation of AP/NTS neuronal activation following cisplatin treatment

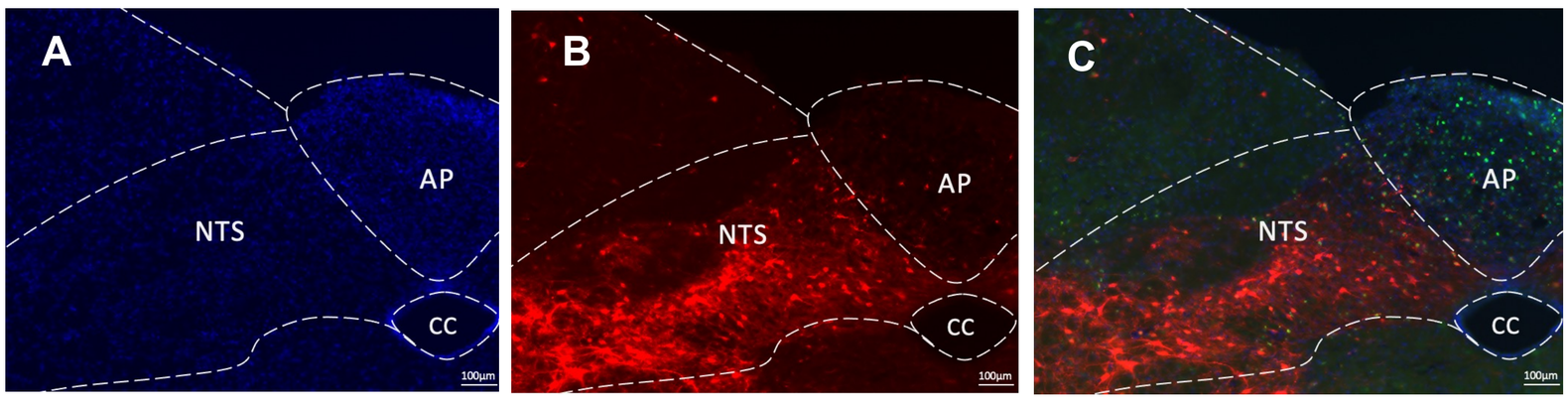

Fig.3: (A) *Representative image of the AP/NTS using the nuclear staining DAPI (blue) in Gad-cre rats.* ***(B)*** *Representative image depicting the spread and intensity of the expression of the excitatory DREADD (Gq) (red).* ***C)*** *Representative immunofluorescent images showing neuronal activation (c-Fos expression; green) in the AP/NTS 24h after CNO (1mg/kg IP) and Cisplatin (6mg/kg IP) combination treatment. Quantification of c-Fos$^+$ neurons in the caudal NTS and in the AP are still ongoing. (Scale bar: 100um).*

Activation of AP/NTS GABA-ergic neurons delays gastric emptying

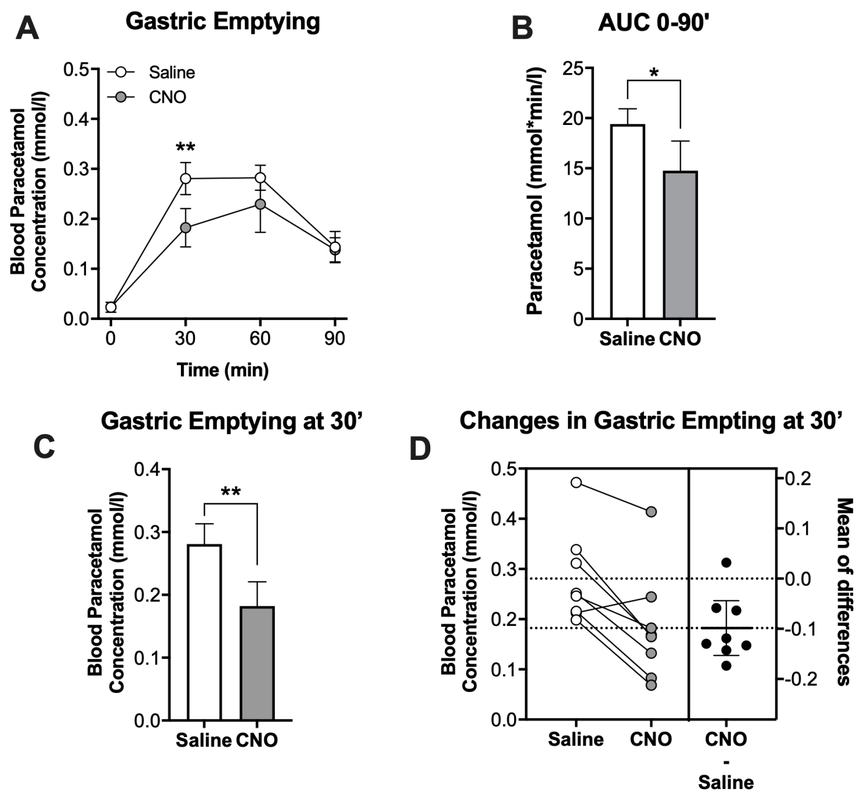

Fig.4: (A) *Changes in circulating paracetamol levels (i.e., a validated proxy for gastric emptying) in response to CNO-induced (1mg/kg IP) activation of AP/NTS GABA-ergic neurons were measured at 0, 30, 60, and 90 min following administration of a liquid meal containing 40mg paracetamol (n = 8 per group).* ***(B)*** *Paracetamol AUC from 0 to 90 min after treatment.* ***(C-D)*** *Blood paracetamol concentrations of the CNO group at 30 min after the administration of the liquid meal was significantly lower than the control group. All data are expressed as mean ± SEM. Data in A were analyzed with repeated-measurements 2-way ANOVA, followed by the Sidak post hoc test. Data in B-D were analyzed with paired t-test. Means with different letters are significantly different from each other (P < 0.05).*

Conclusions

Activation of AP/NTS GABA-ergic neurons attenuated both semaglutide-induced and cisplatin-induced malaise and also increased their hypoghagic effects, partially via delayed gastric emptying.

Our results point to a key role of GABA-ergic neurons as understudied modulators of feeding and illness-like behaviors and provide a neuroanatomical and mechanistic explanation for the anti-emetic effects of GIPR agonisms.

Acknowledgement

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