A Projection from the Hypothalamic Paraventricular Nucleus to the Nucleus Tractus Solitarii is Essential for Cardiorespiratory Responses to Hypoxia

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Introduction: The hypothalamic paraventricular nucleus (PVN) is critical for cardiorespiratory responses to arterial chemoreflex activation. We recently reported that acute hypoxia (Hx) activates PVN neurons that project to the nucleus tractus solitarii (nTS) but the functional role of this projection has not been fully delineated. We hypothesized PVN inputs to the nTS facilitate cardiorespiratory responses to Hx. To test this, male SD rats received bilateral microinjections of retrograde AAV-pgk-Cre into the nTS. One week later, Cre-dependent AAV2-DIO-hSyn-mCherry expressing excitatory (Gq) or inhibitory (Gi) DREADD, or control virus (mCh) was bilaterally microinjected into the PVN, and 3-5 weeks allowed for expression in nTS-projecting PVN neurons.

Objective: To determine the contribution of the PVN to nTS pathway in chemoreflex function.

Method: We assessed ventilatory responses (plethysmography) to progressive Hx (14-8% O_2) in conscious rats before and after ip injection of saline or the synthetic selective DREADD ligand C21 (1mg/kg). We similarly evaluated the role of this pathway in Hx-induced nTS neuronal activation (Fos immunoreactivity, IR); 60 min after ip saline or C21, conscious mCh and Gi rats were exposed to Hx (2 hr, 10% O_2) and Gq rats were exposed to 12% O_2.

Results: Saline had no effect on either ventilatory responses or nTS neuronal activation to Hx in any group. In Gq rats (n=8), DREADD-mediated selective *activation* of nTS-projecting PVN neurons with C21 enhanced ventilatory responses to mild hypoxia (14 and 12% O_2, $p<0.05$). This was associated with increased Hx-induced nTS neuronal activation (377±45 vs. 550±23 Fos-IR cells; saline vs. C21). Conversely, selective *inhibition* of the PVN to nTS pathway by C21 in Gi rats blunted hypoxic ventilatory responses to 10% and 8% O_2 (n=8, $p<0.05$) and produced decreased Hx-induced nTS neuronal activation (581±33 vs. 375±150 Fos-IR cells; saline vs. C21). To confirm that altered cardiorespiratory chemoreflex responses were mediated by PVN terminals in the nTS, anesthetized rats were exposed to brief (45s) Hx episodes (10% O_2, mCh and Gi rats; 12% O_2, Gq rats) before and after bilateral nTS microinjection of C21 (0.1mM;

90nl/side). Mean arterial pressure (MAP), heart rate (HR), splanchnic sympathetic nerve activity (sSNA) and phrenic nerve activity (PhrNA) were measured. Under control conditions, Hx decreased MAP and increased HR, sSNA and PhrNA in all groups. Peak responses to 10% O_2 were similar in mCh and Gi rats and greater than responses to 12% O_2 in Gq rats. nTS microinjection of C21 had minor baseline effects, which were not different among groups. nTS C21 did not alter the Hx-induced increase in PhrNA in mCh rats. In Gq rats (n=3), DREADD-mediated activation of PVN terminals in the nTS enhanced both PhrNA (+51±36%) and sSNA (+166±41%) responses to Hx (12% O2). In contrast, inhibition of PVN terminals in the nTS blunted both PhrNA (-63±10%; $p<0.05$) and sSNA (-32±18%; $p<0.05$) responses to Hx (10% O2) in Gi rats (n=3).

Conclusions: Together, these results suggest that a PVN to nTS pathway directly enhances nTS neuronal activation and cardiorespiratory responses to hypoxia, and is required for responses to more severe hypoxia.

Keywords: acute hipoxia, hypothalamic paraventricular nucleus, cardiorespiratory responses, chemoreflex activation