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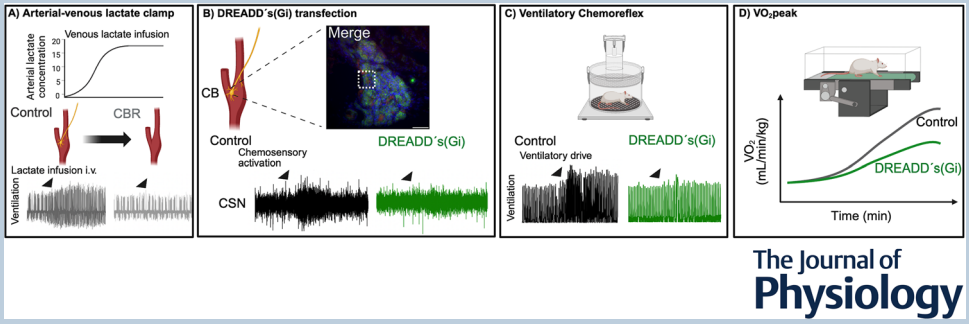
The carotid body mediates peak oxygen uptake during maximal physical exertion in rats

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Abstract figure legend (*A*) Wistar Kyoto rats underwent arterial-venous lactate clamp before and after carotid body resection (CBR). (*B*) After that a viral transfection to express a designer receptor exclusively activated by designer drugs-Gi (DREADD's(Gi)) in CB glomus cells was performed, thereby reducing CB chemosensory activation. (*C*) Besides the activation of DREADD's(Gi) was able to reduce both the hypobaric hypoxic ventilatory drive and (*D*) the peak O_2 uptake ($\dot{V}_{O_2peak}$) during physical exertion. Created in BioRender. https://BioRender.com/ytzjwzt.





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Abstract Cardiorespiratory responses to physical exercise are expected to meet the organism's metabolic demands. As carotid body (CB) glomus cells have been proposed as metabolic sensors, we sought to determine their contribution to peak oxygen uptake ($\dot{V}_{O_2peak}$) during exercise in rats. Adult male Wistar Kyoto rats underwent bilateral co-injection of two adeno-associated viruses (AAVs) at the CB bifurcation (AVV-TH-Cre-SV40 and AVV-hSyn-DREADD(Gi)-mCherry). Clozapine-*N*-oxide (1 mg/kg, I.P.) was administered to activate the inhibitory DREADD-Gi receptor and suppress CB chemosensory activity. Three weeks after AVV infection we evaluated ventilatory and CB chemosensory responses to sodium cyanide (NaCN), the hypobaric-hypoxic ventilatory response (HHVR), lactate-dependent ventilatory response, arterial blood pressure, exercise performance and $\dot{V}_{O_2peak}$. Chemogenetic inhibition of CB glomus cells reduced resting oxygen consumption and ventilatory responses to lactate. In anaesthetized rats acute chemogenetic inhibition of glomus cells markedly diminished the CB chemosensory and ventilatory responses elicited by NaCN, as well as lactate-dependent hyperventilation after CB resection. Similarly HHVR was markedly reduced in non-anaesthetized animals. Notably chemogenetic inhibition of CB glomus cells significantly reduced $\dot{V}_{O_2peak}$ without altering the time required to reach it. These findings support a novel role for CB glomus cells as metabolic sensors that influence $\dot{V}_{O_2peak}$ during maximal physical exertion, independent of overall exercise performance.

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Key points

- Carotid body (CB) glomus cells may function as sensors of metabolic activity through the release of lactate from muscle and its accumulation during physical exertion.
- CB type I chemoreceptor cells are necessary and play a crucial role in sensing metabolism at rest and during exertion.
- The CB acts as a metabolic sensor that triggers metabolism during physical exertion, mediating the increment of peak O_2 uptake during exertion, without affecting exercise performance.

Introduction

During incremental exercise to exhaustion oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) can increase more than 20-fold to meet the body's escalating metabolic demands (Rossiter, 2011). Cardiopulmonary exercise testing (CPET) is the standard method to assess maximal oxygen consumption during exercise (Lee & Zhang, 2021; Rossiter, 2011). At the onset of exercise cardiac output increases, and as intensity progresses both heart rate (HR) and minute ventilation ($\dot{V}_E$) increase progressively, accompanied by mild lactate accumulation (Anrep & Barsoum, 1935; Lee et al., 1982; Luttrell & Halliwill, 2017). With maximal effort these cardiorespiratory (HR and $\dot{V}_E$) and metabolic responses are further elevated, and lactate levels can rise markedly, often exceeding 15 mM in venous blood (Fletcher et al., 2013; Hofmann & Tschakert, 2017).

Recent results suggest that the carotid body (CB) – the primary arterial chemoreceptor for detecting arterial blood changes in pO_2, pCO_2 and pH (Iturriaga & Diaz, 2025; Iturriaga et al., 2021) – also contributes to breathing and haemodynamic regulation in response to elevated lactate levels (Chang et al., 2015; Peng et al., 2021; Torres-Torrelo et al., 2021). Therefore released and/or accumulated lactate during exercise may activate CB chemosensory discharge, in turn stimulating central chemoreflex pathways that initiate or enhance cardiorespiratory and metabolic responses (Iturriaga et al., 2021). However this hypothesis has yet to be experimentally confirmed.

Multiple endogenous signals, including lactate, have been shown to activate CB-mediated chemoreflexes (Chang et al., 2015; Peng et al., 2020; Spiller et al., 2021; Torres-Torrelo et al., 2018). For instance olfactory receptor 78-deficient mice ($Olfr78^{-/-}$) exhibit blunted CB chemosensory responses to hypoxia (10% and 12% O_2) and to 20 mM lactate (Chang et al., 2015; Peng et al., 2020). However some conflicting reports indicate that $Olfr78^{-/-}$ mice

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exhibit normal lactate-dependent ventilatory responses despite decreased hypoxic sensitivity (Peng et al., 2020; Torres-Torrelo et al., 2018). Supporting the role of the CB as a metabolic sensor exogenous lactate has been shown to depolarize isolated CB type I cells via monocarboxylate transporters (MCTs) (Torres-Torrelo et al., 2018, 2021). In contrast other studies in hemisected Wistar rats and isolated CB glomus cells have reported non-respiratory responses to lactate (20 mM), although arterial lactate concentrations were not verified during infusion in *in vivo* experiments – an important methodological limitation (Spiller et al., 2021).

Importantly the actual lactate levels sensed by the CB may differ from those infused systemically due to the rapid lactate clearance metabolic rate (Emhoff & Messonnier, 2023; Juel, 1997), complicating interpretation. Nonetheless prior findings in Wistar rats show robust ventilatory responses to 2 M lactate infusion (Olsson et al., 2002), reinforcing the idea that lactate can modulate CB activity. Despite conflicting evidence the CB is increasingly viewed as a metabolic sensor capable of eliciting lactate-dependent cardiorespiratory responses, which may be relevant during physical exertion (Andrade, 2024).

Additional support for the CB's role in exercise physiology comes from clinical and experimental studies. In asthmatic patients CB resection (CBR) markedly decreased the hypoxic ventilatory response across all CPET phases compared to those with intact CBs (Honda et al., 1979). In rats CBR diminished active inspiration and expiration during steady-state exercise (Spiller et al., 2020). Furthermore the CB has been implicated in sympathetic-mediated blood flow redistribution during exercise (Stickland et al., 2007, 2008, 2011). Low-dose dopamine inhibition of CB activity elicited vasodilatation during exercise in both dogs with heart failure and healthy humans (Sayegh et al., 2024; Stickland et al., 2007, 2011). Given the large-scale release and accumulation of lactate at exhaustion, we hypothesize that the CB acts not only as an oxygen sensor but also as a metabolic sensor that facilitates oxygen uptake during intense exercise. However no studies to date have directly examined this mechanism. Therefore we sought to determine the contribution of CB glomus cells to peak O_2 uptake ($\dot{V}_{O_2peak}$) during exercise in rats using a chemogenetic inhibition approach.

Methods

Ethical approval

Twenty-three male adult Wistar Kyoto rats (∼280 g) were obtained from the Animal Facility at the Universidad de Antofagasta, Antofagasta Region, Antofagasta city, Chile. The rats were housed under controlled temperature and humidity conditions, with a standard 12-h light/dark cycle. They had *ad libitum* access to water and were fed a standard laboratory diet (Prolab RMH3000, LabDiet, St. Louis, MO, USA). All procedures adhere to the animal welfare guidelines of the American Physiological Society and were approved by the Ethics Committee on Scientific Research of the Universidad de Antofagasta (CEIC-UA 365/2022). At the conclusion of the experiments animals were humanely killed via intraperitoneal overdose of sodium pentobarbital (100 mg/kg). Physiological signals were acquired using an analogue-to-digital system (PowerLab/4SP, ADInstruments, Castle Hill, NSW, Australia), and analysed using LabChart Pro 8.0 software.

Animal allocation and experimental groups

The 23 animals were randomly allocated to different experimental procedures. Eight animals were used in arterial-venous lactate clamp experiments to evaluate lactate-driven ventilatory responses. Another group was used to assess hypobaric-hypoxic ventilatory response (HHVR) (non-anaesthetized) and sodium cyanide (NaCN) ventilatory responses (anaesthetized). Within this group four animals were assigned to the sham condition, whereas eight were used to evaluate the effects of chemogenetic inhibition via designer receptors exclusively activated by designer drugs (DREADD(Gi)). From the latter four animals underwent vascular access button implantation for experiments assessing lactate-dependent ventilation and $\dot{V}_{O_2}$ at rest. To assess peak oxygen uptake during exercise a set of eight DREADD(Gi) animals underwent gas exchange testing. Additionally three animals were used to measure carotid sinus nerve (CSN) chemosensory activity and for immunohistochemical validation of viral transfection. A summary of the experimental distribution is provided in Fig. 1.

Experimental procedure

To test our hypothesis three sets of experiments were conducted. First to determine whether lactate can elicit a ventilatory response we performed an arterial-venous lactate clamp (Fig. 2). Second in a separate series one CB per animal was transfected with adeno-associated virus (AAV) vectors to express the inhibitory DREADD(Gi) receptor. Clozapine-*N*-oxide (CNO, 1 mg/kg) was administered intraperitoneally to suppress CB chemosensory activity, and ventilatory responses to NaCN were assessed. The contralateral CB served as an internal control. At the end of these experiments the transfected CB was extracted and processed for immunohistochemical localization of mCherry and tyrosine hydroxylase (TH) (Fig. 2). Third *in vivo* physiological experiments were performed after 3 weeks of viral trans-

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fection or sham surgery to measure $\dot{V}_{O_2peak}$, HHVR and NaCN-evoked cardiorespiratory responses. Additionally 2 weeks post-transfection four of the DREADD(Gi) animals underwent vascular access button implantation to assess whether CB glomus cells are required to mediate lactate-dependent metabolic responses at rest (Fig. 2).

Arterial-venous lactate clamp

Surgical procedure. To perform the arterial-venous lactate clamp before and after CBR (Andrade et al., 2021b; Del Rio et al., 2013, 2016) animals were anaesthetized intraperitoneally with α-chloralose (40 mg/kg) and urethane (1.2 g/kg) (Sigma-Aldrich, St. Louis, MO, USA) (Maia et al., 2015). Anaesthesia depth was confirmed via tail pinch and the absence of a withdrawal reflex response. Once adequate anaesthesia was confirmed, animals were placed in a supine position on a warming pad (Kent Scientific, Torrington, CT, USA) to maintain body temperature at 38.0 ± 0.5°C. A midline neck incision was made to expose and catheterize the jugular vein using PE-50 polyethene tubing (Becton Dickinson and Company, Franklin Lakes, NJ, USA) for venous lactate infusion. The trachea was cannulated with a flexible tube to allow ventilation. Additionally the right femoral artery was exposed and cannulated (PE-10 tubing) for arterial blood sampling to measure lactate concentration during lactate infusion. At the conclusion of the experiments animals were killed with an overdose of sodium pentobarbital (100 mg/kg, I.P.).

Ventilation recording. The tracheal cannula was connected to a Spirometer Pod for real-time airflow recording. Ventilatory variables, including tidal volume (V_t), respiratory frequency (R_f) and minute ventilation ($\dot{V}_E = V_t \cdot R_f$), were calculated using LabChart software, as previously described (Rey et al., 2006).

Lactate infusion and measurement. After the surgery and catheterization, animals were allowed a 20-min stabilization period. A 1.2 M sodium lactate solution was then infused intravenously at a rate of 60 µL/min for 6

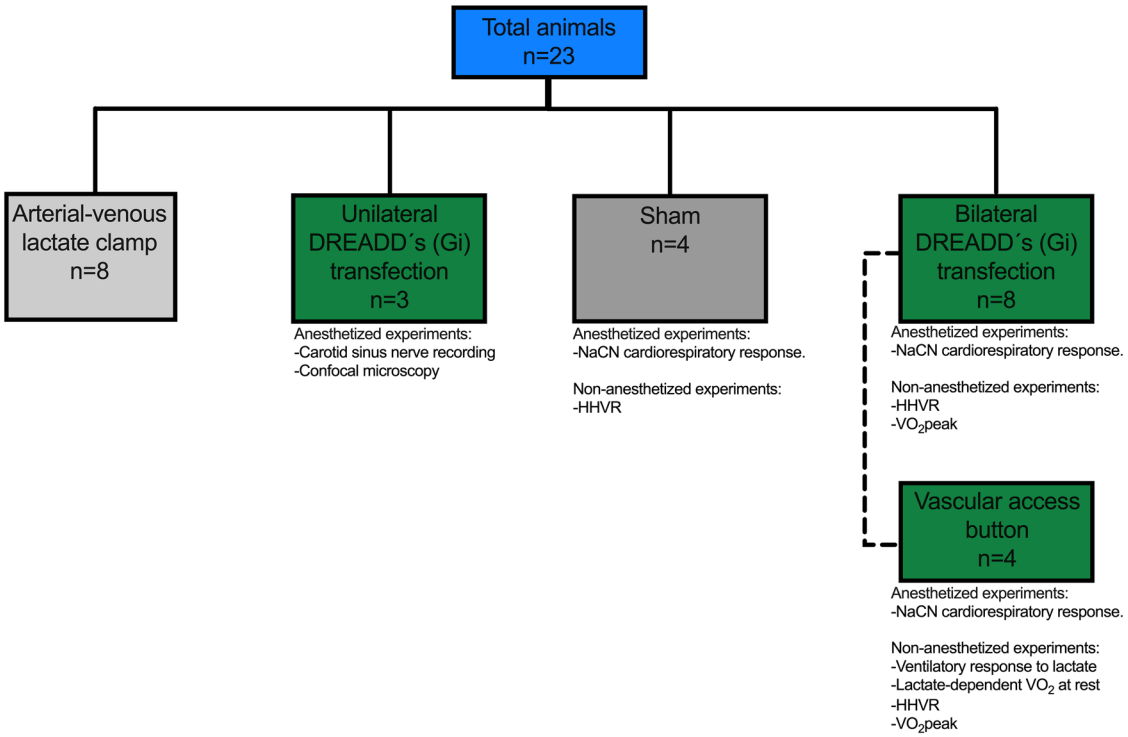


Figure 1. Flowchart of animal allocation and experimental groups
The study began with 23 male adult Wistar Kyoto rats, of which n = 8 underwent an arterial-venous lactate clamp. In a separate experiment n = 3 rats underwent unilateral carotid body (CB) transfection with a designer receptor exclusively activated by designer drugs-Gi (DREADD(Gi)), and carotid sinus nerve (CSN) activity was assessed from the transfected and non-transfected contralateral sides of the same animal. Four sham animals (n = 4) were used to determine the NaCN and hypobaric-hypoxic ventilatory responses (HHVR). In another animal experimental series the CBs from eight rats were bilaterally transfected with DREADD(Gi). One week prior to the physiological experiments n = 4 rats underwent vascular access port implantation. The physiological experiments were NaCN ventilatory response, HHVR, lactate-dependent ventilatory response and O_2 uptake at rest and peak O_2 uptake during exercise.

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min. Arterial blood samples (∼50 µl) were collected from the right femoral artery at baseline and during infusion (at 2, 4 and 6 min). From each sample 10 µl was applied to a reactive test strip compatible with the Lactate Pro 2 analyser (Arkray, Kyoto, Japan) to quantify arterial lactate concentration. The clamp protocol was repeated after CBR to compare ventilatory and metabolic responses under both conditions.

Virus infection and DREADD(Gi) expression

VitroGel hydrogel was mixed 1:1 (v/v) with the virus suspension and pipetted gently 5–10 times to ensure thorough mixing. The mixture consisted of 2 µl of an AAV vector encoding a Gi-coupled hM4D DREADD fused with mCherry under the human synapsin promoter (AAV9-hSyn-DIO-hM4D(Gi)-mCherry; 1×10^{13} – 7×10^{12} vg/ml, Addgene cat# 44362) and 2 µl of a

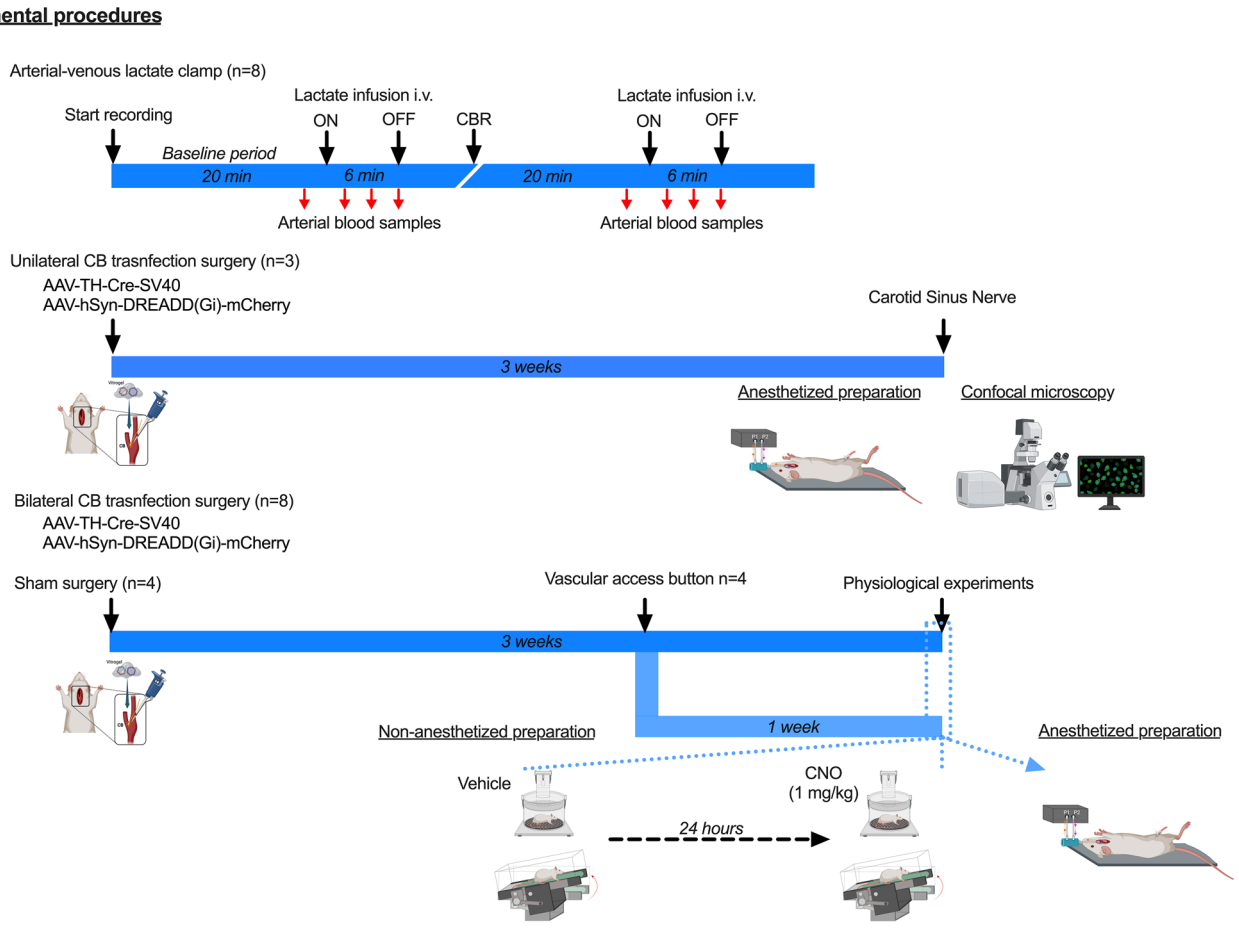


Figure 2. Experimental design and timeline experiments
The current study consisted of three separate experiments. *The* first experiment consisted of an arterial-venous lactate clamp (*n* = 8). The ventilatory recording began at time 0, and the baseline period lasted 20 min. After that a venous lactate infusion was initiated for 6 min, during which arterial blood samples were collected every 2 min to assess arterial lactate concentration. At the end of the lactate infusion the carotid bodies were resected, and after a new 20 min baseline period the lactate infusion and blood samples were repeated. The second experiment consisted of a unilateral CB transfection with a designer receptor exclusively activated by designer drugs-Gi (DREADD(Gi)) to assess whether clozapine-*N*-oxide (CNO) can reduce the carotid sinus nerve (CSN) activity during NaCN administration. At time 0 three animals underwent surgery to transfect one CB, and the contralateral side was subjected to the same procedure, but without transfection, which served as their control. After 3 weeks, the CSN was recorded from the transfected and non-transfected CB. After that the CBs from transfected animals were surgically removed and fixed for immunolocalization of mCherry in tyrosine hydroxylase–positive cells. During the third experiment *n* = 12 animals were subjected to a surgery for CB bilateral transfection (*n* = 8) or sham (*n* = 4). One week prior to physiological experiments four rats (*n* = 4) underwent a second surgery to implant a vascular access button for drug administration. After 3 weeks post-transfection or sham surgery the animals underwent physiological experiments before and after CNO administration.

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Cre-expressing AAV driven by the rat tyrosine hydrolase (rTH) promoter (AAV9.rTH.PI.Cre.SV40; 1×10^{13} vg/ml, Addgene cat# 107788), mixed with 4 µl of Vitrogel (The Well Bioscience, SKU: TWG001). The hydrogel mixture was stabilized on ice or at 4°C for 5–10 min before bilateral application to the CBs of eight rats. In an additional group ($n = 3$) the virus-hydrogel mixture was applied unilaterally to one CB. CNO (1 mg/kg, I.P.) was administered to activate the DREADD(Gi) and inhibit CB glomus cell activity.

Immunofluorescence and DREADD's expression

Three rats were killed for immunohistochemical analysis of DREADD expression. Carotid bifurcations containing the CB were dissected and fixed overnight at 4°C in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). The following day tissues were cryoprotected by immersion in a sucrose gradient (10%, 20% and 30% in PBS) until saturated, embedded and sectioned at 30 µm using a cryostat. Free-floating sections were rinsed thrice in 1× PBS and permeabilized in a solution of PBS containing 0.2% Triton X-100. After blocking in 1× PBS containing 3% bovine serum albumin (BSA) for 1 h at room temperature sections were incubated overnight at 4°C with a rabbit anti-TH antibody (1:1000; ELK Biotechnology) diluted in blocking buffer. After three PBS washes (5 min each) sections were incubated for 2 h at 37°C with Alexa Fluor 555-conjugated anti-rabbit secondary antibody (1:200; Invitrogen). Nuclei were counterstained with DAPI (3 µL in distilled water), and sections were mounted with DAKO fluorescence mounting medium. Images were acquired at 40× magnification using a Confocal Spectral Laser Scanning Microscope (Olympus FV1000 LSM, Tokyo, Japan) equipped with standard laser lines (405, 488, 543 and 633 nm). Z-stacks were collected at 1 µm intervals. Image processing and fluorescence quantification were performed using Fiji (ImageJ, NIH).

Surgical procedure

Virus transfection. A total of 11 rats were used for viral transfection. Animals were anaesthetized with 5% isoflurane in 21% O_2 using a specialized induction chamber (RWD, China). Anaesthesia depth was confirmed by tail pinch and absence of a withdrawal reflex. Animals were then placed on a warming pad (Kent Scientific) to maintain body temperature at 38.0 ± 0.5°C and maintained under anaesthesia with 2% isoflurane in 21% O_2. A midline cervical incision was made to expose the carotid bifurcation and isolate both CBs. The prepared VitroGel/virus mixture was loaded into a micropipette and gently applied to the CB bifurcation. In one group ($n = 8$) the virus was applied bilaterally, and in another group ($n = 3$) it was applied unilaterally. After a 15-min stabilization period the incision was sutured using 3-0 absorbable thread (Tagum, Lima, Peru). Postoperative care included subcutaneous administration of ketoprofen (1%) for analgesia and enrofloxacin (5%) as an antibiotic.

Vascular access button implantation. Two weeks after transfection four of the eight bilaterally infected animals ($n = 4$) underwent a second surgery to implant a vascular access button, following previously established procedures (Del Rio et al., 2013; Toledo et al., 2017). Anaesthesia was induced with 5% isoflurane in 21% O_2 using a specialized induction chamber (RWD, China). Once anaesthetic depth was confirmed by the absence of a withdrawal reflex to tail pinch, animals were maintained under anaesthesia with 2% isoflurane in 21% O_2, with body temperature held at 38.0 ± 0.5°C using a warming pad (Kent Scientific). A midline neck incision was made to isolate a lateral branch of the jugular vein. A 3Fr polyurethane catheter was inserted into the jugular vein and tunnelled subcutaneously to the dorsal surface of the neck, where it was connected to a vascular access port. The fat and skin layers were sutured with a 3-0 absorbable thread (Tagum, Lima, Peru). Subcutaneous analgesics (ketoprofen 1%) and antibiotics (enrofloxacin 5%) were administered postoperatively.

NaCN cardiorespiratory responses during acute chemogenetic CB glomus cells inhibition

To assess the effects of acute chemogenetic inhibition of CB glomus cells on cardiorespiratory responses animals from the sham ($n = 4$) DREADD(Gi) ($n = 8$) groups were anaesthetized with α-chloralose (40 mg/kg, I.P.) and urethane (1.2 g/kg, I.P.) (Sigma-Aldrich), as described previously (Maia et al., 2015). Anaesthetic depth was verified by the absence of reflex withdrawal to tail pinch. Animals were positioned supine on a warming mat (Kent Scientific) and maintained at a constant body temperature of 38.0 ± 0.5°C.

A midline neck incision was made to expose and cannulate the trachea for airflow recording and to isolate and catheterized the jugular vein for NaCN administration (100 µg/kg in 100 µl). A small incision in the left hind limb was made to isolate and cannulate the femoral artery for continuous blood pressure monitoring. Blood pressure was measured using a pressure transducer connected to a data acquisition system (AD instruments). From the raw arterial pressure signal systolic (SBP) and diastolic (DBP) pressures were recorded, and pulse pressure (PP = SBP − DBP) and mean arterial pressure (MABP = 1/3 SBP + 2/3 DBP) were calculated. Heart rate (HR) was derived from the first derivative of blood pre-

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ssures signal (dP/dt), as described previously (Andrade et al., 2017; Del Rio et al., 2016). Ventilatory variables, including tidal volume ($\dot{V}_t$), respiratory frequency (R_f) and minute ventilation ($\dot{V}_E = \dot{V}_t \times R_f$), were recorded by connecting the tracheal cannula to a Spirometer Pod (Rey et al., 2006). Responses to NaCN were assessed before and after the administration of CNO (1 mg/kg, I.P.).

Carotid sinus nerve recording

In unilaterally transfected animals ($n = 3$) CSN discharge was recorded from both the transfected and non-transfected CBs in the same animal using an *in situ* preparation, as previously described (Andrade et al., 2018; Del Rio et al., 2013; Pijacka et al., 2016). Anaesthesia was induced intraperitoneally with α-chloralose (40 mg/kg) and urethane (1.2 g/kg) (Maia et al., 2015), and depth of anaesthesia was confirmed by the absence of withdrawal reflex to tail pinch. Animals were placed in the supine position on a warming pad to maintain body temperature at 38.0 ± 0.5°C. A midline cervical incision was made to expose the trachea, which was then cannulated with a flexible tube. Both CBs were visualized. The right CSN was isolated and placed on a pair of iridium-platinum electrodes connected to a NeuroAmp headstage (ML185) and covered with warm mineral oil to ensure stable electrical contact. The nerve signal was visualized using an oscilloscope (Protek 6502, Taiwan), amplified (DP301, Warner Instruments, USA) and filtered (bandpass 30–500 Hz). Dopamine (10 µg/kg/min, I.V.) was used to verify the threshold of CB sensory activity, as previously described (Pijacka et al., 2016). The maximal chemosensory response was evoked by a bolus of NaCN (100 µL, 100 µg/kg, I.V.), and recordings were obtained before and after CNO administration (1 mg/kg, I.P.). The same procedure was then repeated on the contralateral (non-transfected) CB, allowing each animal to serve as its control. Signals were acquired using a PowerLab data acquisition system and analysed with LabChart Pro 8.0 software.

Whole-body plethysmography

Ventilatory variables (V_t, R_f and $\dot{V}_E$) were measured using a custom-built, unrestrained whole-body plethysmography in sham ($n = 4$) and DREADD(Gi) ($n = 8$) animals, as previously described (Del Rio et al., 2013; Díaz et al., 2020; Toledo et al., 2017, 2019), before and after CNO administration (1 mg/kg, I.P.). The plethysmograph chamber had a total volume of 2 l. Two pumps were used to control inflow at 2 l/min (Air Supply and Switching, PanLab Harvard Apparatus, Spain) and outflow at 1.5 l/min (Vent2, Emka Technologies, France). All recordings were conducted at ambient temperature (25 ± 2°C).

Resting ventilation was measured in normobaric normoxia (NN) while the rats breathed room air (Del Rio et al., 2013; Díaz et al., 2020; Toledo et al., 2017, 2019). After a 20 min baseline recording under NN conditions (PO_2 ∼156 mmHg) the peripheral chemoreflex was stimulated using hypobaric hypoxia (HH) in a hypobaric hypoxic chamber (Genetic Core, Peru), simulating an altitude of 5500 m (PO_2 ∼60 mmHg). The ascent was performed at a rate of 6.4 mmHg/min, equivalent to a speed ascent of 366.6 m/min (Salazar-Ardiles et al., 2024). Animals remained at peak altitude for 10 min. A camera (Spl6506bm, Philips, Netherlands) was used to identify and exclude breathing segments with movement artifacts. For each animal the average of three artifact-free segments during NN and HH was used to analyse the HHVR.

The same plethysmography set-up was used to assess lactate-dependent metabolic and ventilatory responses. In these experiments lactate (1.2 M) was infused via a previously implanted vascular access port in the jugular vein (Del Rio et al., 2016). Additionally during these recordings the outflow circuit (Vent2) was connected to a gas analyser to measure oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) before and during lactate infusion.

Gas exchange during an incremental exercise test

Rats ($n = 8$) underwent an incremental exercise test with gas exchange measurement before and after silencing CB glomus cells with CNO. The test was conducted on a single-lane, motor-driven treadmill for rodents. Oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) were calculated as previously described (Jeton et al., 2017; Marcouiller et al., 2014):

$$VO_2 \text{ consumption} = \text{Flow} \cdot \frac{[(F_iO_2 - F_eO_2) - F_eO_2 \cdot (F_eCO_2 - F_iCO_2)]}{(1 - F_eO_2)} \quad (1)$$

$$VCO_2 \text{ production} = \text{Flow} \cdot \frac{[(F_eCO_2 - F_iCO_2) - F_eO_2 \cdot (F_iO_2 - F_eO_2)]}{(1 - F_eCO_2)} \quad (2)$$

where F_i and F_e refer to the fractional concentrations of O_2 and CO_2 in the inflow and outflow, respectively. F_iO_2 and F_iCO_2 correspond to the gas composition of ambient room air. Inspired and expired O_2 and CO_2 concentrations were sampled every 3 min using a gas analyser (LE406 system Gas Analyzer, PanLab Harvard Apparatus, Spain). The analyser was calibrated prior to each session with certified reference gas (low point: O_2 = 20.5%, CO_2 = 0.0%; high point: O_2 = 50.6%, CO_2 =

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1.5%). Both inflow and outflow rates were maintained at 1 l/min. The treadmill protocol began at 41.7 cm/s with a 2° incline, increasing speed by 5 cm/s every minute. A mild electrical stimulus (0.5 mA) at the rear of the treadmill was used to encourage continued running. The test was terminated when the animal showed signs of exhaustion, defined as remaining on the electrical grid for 10 s without re-engaging the treadmill belt (Salazar-Ardiles et al., 2024). Exercise performance was quantified by the maximum aerobic speed, defined as the treadmill speed reached at $\dot{V}_{O_2peak}$. The respiratory quotient (RQ) was calculated as the ratio of CO_2 production to O_2 consumption (Beaver et al., 1986). All gas exchange signals were sampled at 1 kHz and acquired using a PowerLab/4SP analogue-to-digital system.

Statistical analysis

All data are presented as mean ± SD, with individual data points displayed as dots in the figures. Normality of each dataset was assessed using the Shapiro-Wilk test, and the corresponding W- and *P*-values are shown in every figure. Comparisons between conditions were performed using a two-way repeated-measures ANOVA (2×2 design), followed by Holm-Sidak *post hoc* tests conducted separately for sham and DREADD(Gi) groups. The Δ differences were assessed using paired or unpaired two-tailed *t* tests, depending on the structure of the data. A *P*-value <0.05 was considered statistically significant. Exact *P*-values are reported in all figures and tables for every statistically significant difference. Sample sizes for each dataset are indicated in the figure legend and table captions. All statistical analyses were performed using GraphPad Prism software (version 10.0, La Jolla, CA, USA).

Results

Ventilatory responses to lactate

To determine whether lactate elicits a CB-dependent ventilatory response we performed an arterial-venous lactate clamp before and after the CBR in the same animal preparation (Fig. 3*A*). Arterial lactate concentrations during infusion were similar before and after CBD (16.2 ± 4.3 *vs.* 16.5 ± 4.6 mM, Control *vs.* CBR, $n = 8$; Fig. 3*B*). Lactate infusion induced significant increases in tidal volume (V_t: +Δ 34.7 ± 73.6%), respiratory frequency (R_f: +24.7 ± 45.6%) and minute ventilation ($\dot{V}_E$: +55.6 ± 92.2%) compared to baseline (Fig. 3*C*–*F*). These responses were markedly attenuated after CBR (V_t: +6.1 ± 38.6%; R_f: +2.1 ± 24.6%; $\dot{V}_E$: +6.8 ± 41.4%; Fig. 3*C*–*F*).

To assess individual variability in ventilatory response to lactate we analysed $\dot{V}_E$ changes for each animal. All rats exhibited ventilatory hyperreflexia in response to lactate (range: +12.9% to +109.7%), which was significantly blunted after CBR (range: −8.4 to +34.9%) (Fig. 3*G*). These results suggest that the CB sense lactate at concentrations comparable to those observed during physical exhaustion, eliciting a robust ventilatory response.

Acute chemogenetic inhibition of CB glomus cells reduces the ventilatory response to NaCN

Three weeks after AAV transfection immunofluorescence confirmed DREADD(Gi) expression in CB glomus cells by co-localization of mCherry with tyrosine hydrolase positive (TH^+) cells (Fig. 4*A*). We then evaluated whether CNO suppressed afferent CB chemosensory activity. In non-transfected CBs CNO had no significant effect on the NaCN-evoked CSN discharge (+37.3 ± 2.2 *vs.* +41.3 ± 11.7%, CNO vs. Veh; Fig. 4*B*, left panel). In contrast DREADD(Gi)-transfected CBs showed a marked reduction in NaCN-evoked CSN activity following CNO administration (+8.9 ± 13.2 *vs.* +69.3 ± 8.0%, CNO vs. Veh; Fig. 4*B*, right panel).

We next assessed whether efferent CB drive, as reflected in ventilatory responses to NaCN, was affected by CNO administration. In sham animals NaCN induced increases in R_f (+15.8 ± 26.3%), V_t (+78.8 ± 23.5%) and $\dot{V}_E$ (+70.2 ± 69.3%) that were not significantly affected by CNO (R_f: +25.3 ± 31.1%; V_t: +45.8 ± 44.4%; and $\dot{V}_E$: +90.3 ± 48.8%) (Fig. 4*C* and *D*; Table 1). In DREADD(Gi) animals NaCN also induced robust ventilatory responses (R_f: +31.3 ± 15.4%; V_t: +22.7 ± 56.0%; $\dot{V}_E$: +51.3 ± 45.8%) under vehicle conditions (Fig. 4*E* and *F*; Table 1). However during CNO administration both R_f and $\dot{V}_E$ responses were significantly reduced compared to vehicle (R_f: 113.6 ± 15.5 *vs.* 131.3 ± 15.4% basal; and $\dot{V}_E$: 106.8 ± 32.8 *vs.* 151.3 ± 45.8%) (Fig. 4*E* and *F*; Table 1). Interestingly we observed a compensatory effect in DREADD(Gi) animals during CNO administration, where an increase in baseline V_t was accompanied by a reduction in R_f, resulting in preserved $\dot{V}_E$ under anaesthesia (Table 1).

To assess whether these effects extended to cardiovascular function we recorded blood pressure responses during NaCN administration. In sham animals in the vehicle condition NaCN increased SBP, DBP, MABP and HR. During CNO administration only SBP and HR remained increased (Table 1). In DREADD(Gi) animals NaCN similarly elevated SBP, DBP, MABP and HR in the vehicle condition, but CNO significantly blunted these responses. Specifically the CNO condition showed significantly lower SBP, DBP and MABP increases compared to the vehicle (Table 1), confirming a reduction in CB-mediated cardiovascular reflex responses following chemogenetic inhibition.

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Effect of chemogenetic silencing of CB glomus cells on the hypobaric-hypoxic ventilatory response

After confirming that chemogenetic inhibition of CB glomus cells reduced NaCN-evoked ventilatory responses we evaluated the HHVR in non-anaesthetized animals (Figs 5 and 6). In sham animals R_f and $\dot{V}_E$ (Fig. 5*B–D*) were significantly elevated during HH compared to NN under both vehicle (R_f: +58.6 ± 12.6%; $\dot{V}_E$: +69.8 ± 59.7%) and CNO (R_f: +60.6 ± 19.6%; $\dot{V}_E$: +65.7 ± 38.6%),

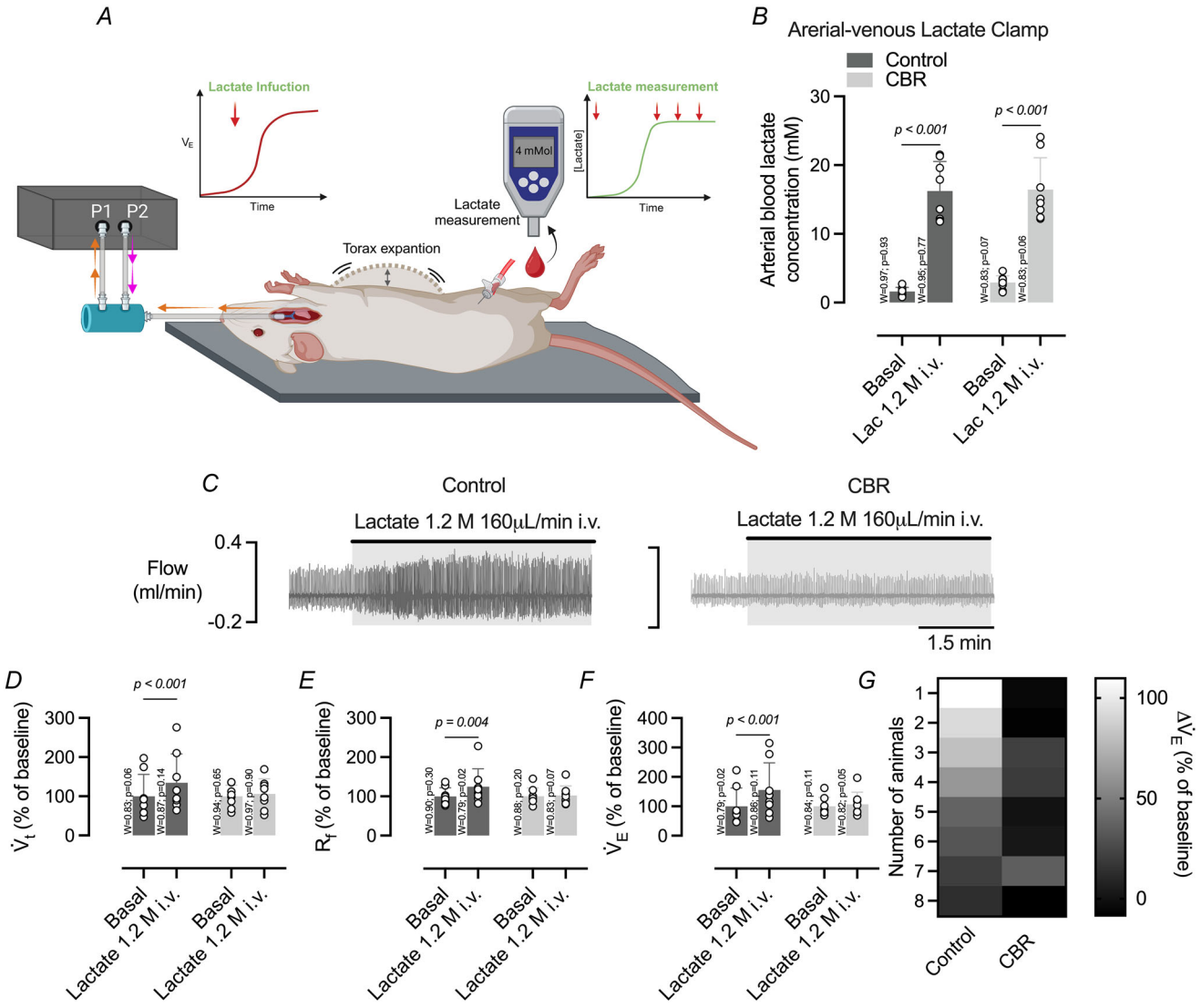


Figure 3. Arterial-venous lactate clamp
A, schematic representation of arterial-venous lactate clamp in anaesthetized preparation. Under the supine position the jugular vein, trachea and both femoral arteries were cannulated. The lactate was infused from the jugular vein, the tracheal cannula was connected to a spirometry pod and blood samples were taken from the femoral artery to measure arterial lactate concentration. *B*, intravenous lactate infusion of 1.2 M elicited a similar arterial lactate level in both groups (Control vs. carotid body resection (CBR)) (∼16 mM), mimicking metabolic stress (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 14)= 191, $P < 0.001$). *C*, representative ventilatory recordings at rest and during lactate infusion in control and CBR conditions. Note that CBR can diminish lactate-dependent hyperventilation. *D*, respiratory frequency (R_f) (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 14) = 7, $P = 0.02$). *E*, tidal volume (V_t) (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 14) = 15, $P < 0.01$) and (*F*) minute ventilation ($\dot{V}_E$) (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 14) = 20, $P < 0.001$) were significantly increased during lactate infusion, and CBR was able to blunt the hyperventilation elicited by lactate. *G*, interindividual variability of the $\Delta\dot{V}_E$ before and after CBR. Note that all animals respond to lactate infusion. The normality assumption was performed with the Shapiro-Wilk test, and the W- and *P*-values for every dataset are shown on the left side of every bar.

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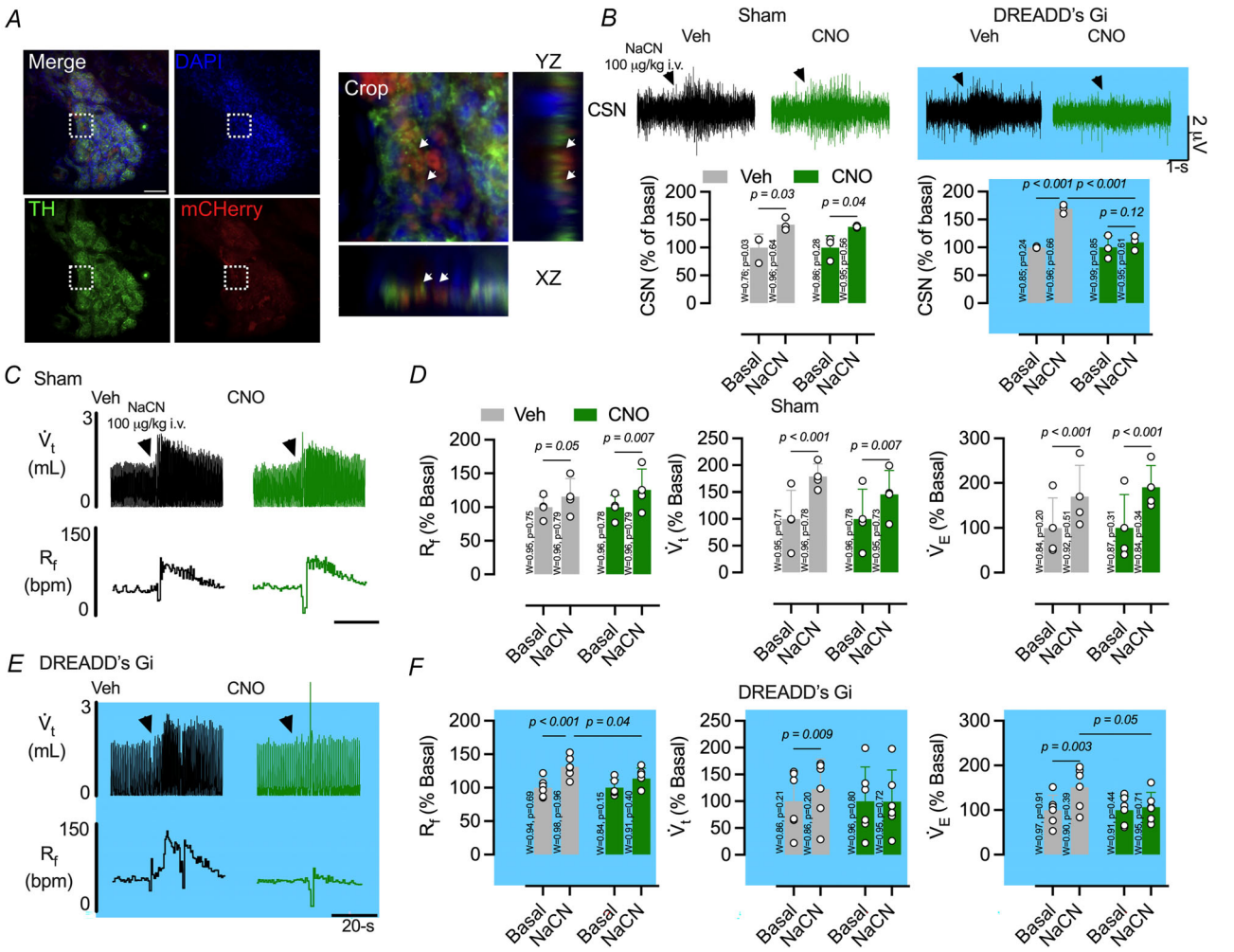


Figure 4. NaCN-dependent hyperventilation during silencing of the carotid body (CB) glomus cells
A, a representative confocal immunostaining of the reporter fluorescence protein, mCherry, TH and DAPI demonstrates the DREADD's transfection. The scale bar represents 200 μm. Arrowhead indicates co-localization of TH glomus cells and DREADDs in 2D and Z-plane, $n = 3$. *B*, representative carotid sinus nerve (CSN) recording during NaCN administration in one sham and DREADD(Gi) animal during vehicle and clozapine-*N*-oxide (CNO, 1 mg/kg, I.P.) administration. NaCN elicited an increment of CSN, which was reduced during CNO administration only in DREADD(Gi) animals (upper panel). The lower panel showed the summary data of CSN in sham (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 4) = 20, $P = 0.01$) and DREADD´s(Gi) (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 4) = 144, $P < 0.001$) rats ($n = 3$). *C*, representative ventilatory traces of tidal volume (V_t) and respiratory frequency (R_f) during NaCN administration before and after CNO administration in sham animals. Note that CNO does not modify the ventilatory response to NaCN. *D*, percentage increase in NaCN-dependent R_f (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 6) = 20, $P < 0.01$), V_t (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 6) = 59, $P < 0.001$) and $\dot{V}_E$ (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 6) = 120, $P < 0.001$) responses before and after CNO administration in sham animals ($n = 4$). Note that the ventilation was significantly increased during NaCN administration to the same extent in vehicle (Veh) and CNO. *E*, representative ventilatory traces of tidal volume (V_t) and respiratory frequency (R_f) during NaCN administration before (Veh) and after CNO administration in animals transfected with DREADD(Gi). Note that CNO markedly reduced the ventilatory response to NaCN. *F*, NaCN-dependent R_f (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn =1, DFd = 10) = 25, $P < 0.001$), V_t (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 10) = 4.9, $P < 0.05$) and $\dot{V}_E$ (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 10) = 10, $P < 0.01$) responses before (Veh) and after CNO administration in DREADD's(Gi) animals ($n = 8$). Note that R_f was significantly increased during NaCN in both conditions; however the NaCN response was significantly reduced during CNO compared to the Veh condition. Notably the NaCN-dependent V_t and $\dot{V}_E$ responses were blunted during CNO administration; moreover $\dot{V}_E$ during NaCN was significantly lower in the CNO condition compared to the NaCN Veh condition. The normality assumption was performed with the Shapiro-Wilk test, and the W- and *P*-values for every dataset are shown on the left side of every bar.

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Table 1. Cardiorespiratory response to NaCN in sham and DREADD(Gi) animals during Veh and CNO administration in an anaesthetized preparation

	Sham ($n = 4$)				DREADD(Gi) ($n = 8$)			
	Veh		CNO (1 mg/kg)		Veh		CNO (1 mg/kg)	
	Baseline	NaCN	Baseline	NaCN	Baseline	NaCN	Baseline	NaCN
SBP (mmHg)	109.7 ± 15.1	116.8 ± 12.9 *$P = 0.001$	108.8 ± 10.9	112.5 ± 11.8 *$P = 0.023$	109.4 ± 12.4	121.2 ± 8.7 *$P = 0.0005$	99.6 ± 9.1	104.6 ± 9.5 +$P = 0.003$
DBP (mmHg)	83.5 ± 15.5	88.0 ± 13.2 *$P = 0.024$	79.0 ± 12.1	82.2 ± 13.2	70.7 ± 16.4	80.3 ± 14.1 *$P = 0.003$	58.6 ± 12.2	61.5 ± 10.5 +$P = 0.009$
MABP (mmHg)	91.3 ± 15.2	96.6 ± 12.9 *$P = 0.008$	88.1 ± 11.6	91.4 ± 12.5	82.7 ± 14.6	92.9 ± 11.5 *$P = 0.0013$	71.6 ± 10.3	75.1 ± 8.9 +$P = 0.004$
HR (beats/min)	358.9 ± 25.1	369.7 ± 25.7 *$P = 0.004$	343.5 ± 10.3	353.8 ± 13.3 *$P = 0.005$	287.9 ± 66.0	305.9 ± 54.9 *$P = 0.0005$	309.6 ± 645	314.8 ± 62.6
V_t (ml/100 g)	0.41 ± 0.1	0.56 ± 0.1 *$P = 0.0003$	0.40 ± 0.1	0.49 ± 0.1 *$P = 0.005$	0.37 ± 0.2	0.46 ± 0.2 *$P = 0.004$	0.45 ± 0.3	0.44 ± 0.3
R_f (breaths/min)	94.5 ± 15.6	109.5 ± 24.8 *$P = 0.045$	92.6 ± 16.5	116.1 ± 28.9 *$P = 0.007$	99.7 ± 13.6	130.8 ± 15.4 *$P < 0.0001$	85.7 ± 10.2 †$P = 0.042$	97.4 ± 13.3 *$P = 0.036$ +$P < 0.0001$
$\dot{V}_E$ (ml/min/100 g)	38.8 ± 14.8	61.4 ± 25.3 *$P = 0.007$	37.6 ± 14.1	57.0 ± 26.4 *$P = 0.012$	37.5 ± 22.1	61.5 ± 29.5 *$P = 0.0001$	38.6 ± 24.7	43.6 ± 24.2

Note: Data are shown as mean ± SD. The data were analysed using a repeated-measures ANOVA (2 × 2) followed by the Holm-Sidak *post hoc* test.
Abbreviations: CNO, clozapine-*N*-oxide; DBP, diastolic blood pressure; DREADD(Gi), designer receptors exclusively activated by designer drugs transfected animals; HR, heart rate; MABP, mean arterial blood pressure; V_t, tidal volume; R_f, respiratory frequency; SBP, systolic blood pressure; $\dot{V}_E$, minute ventilation; Veh, NaCl 0.9%.
*significant *vs*. baseline (same group).
+significant *vs*. Veh NaCN.
†significant *vs*. Veh baseline.

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without changes in tidal volume (V_t) (Fig. 5*B*–*D*; Table 2). The relative increases (Δ) in ventilatory variables (ΔR_f, ΔV_t and $\Delta \dot{V}_E$) were compared between vehicle and CNO conditions (Fig. 6*A*–*C*).

In DREADD(Gi) animals HH induced significant increases in R_f (+47.4 ± 13.0%) and $\dot{V}_E$ (+59.1 ± 102.4%) compared to NN under vehicle conditions, with no significant effect on V_t (Fig. 5*B*–*D*; Table 2). However CNO administration markedly blunted the HH-induced ventilatory response. $\dot{V}_E$ increased only modestly ($\dot{V}_E$: +16.8 ± 51.2%) and did not reach statistical significance (Fig. 5*D*; Table 2). Furthermore comparisons between vehicle and CNO in DREADD(Gi) animals revealed a significantly less increase in R_f (133.7 ± 19.4 *vs.* 148.3 ± 12.1%), $\dot{V}_t$ (88.4 ± 16.2 *vs.* 108.0 ± 19.3%) and $\dot{V}_E$ (116.8 ± 51.2 *vs.* 159.1 ± 102.4%) during HH under CNO administration (Fig. 5*B*–*D*).

These findings were further confirmed by analyses of individual percentage changes from normoxia to hypoxia (ΔHH–NN). In this approach the ventilatory response to HH was calculated for each animal as a percentage increase from its own NN baseline. Compared to DREADD(Gi) under vehicle conditions CNO administration significantly reduced ΔR_f (33.7 ± 19.4 *vs.* 48.3 ± 12.1%; Fig. 6*A*), ΔV_t (−11.4 ± 16.2 *vs.* 8.0 ± 19.3%; Fig. 6*B*) and $\Delta \dot{V}_E$ (19.6 ± 32.8 *vs.* 58.9 ± 22.9%;

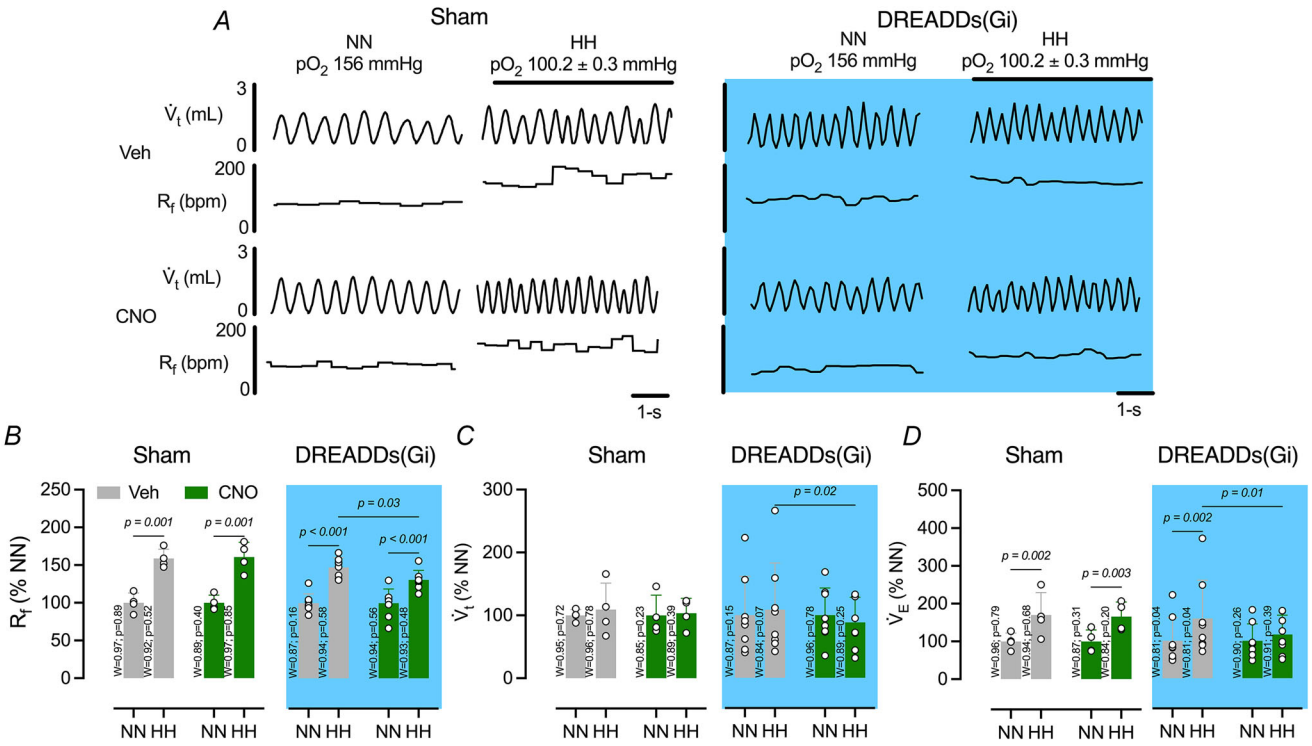


Figure 5. Hypobaric-hypoxic ventilatory response (HHVR) during inhibition of carotid body (CB) glomus cells
A, representative ventilatory traces in normobaric normoxia (NN) and hypobaric hypoxia (HH) during vehicle (Veh) and clozapine-*N*-oxide (CNO, 1 mg/kg, I.P.) administration in non-anaesthetised preparation. Note that sham animals displayed similar HHVR during Veh and CNO administration; however DREADD's(Gi) rats showed a comparable HHVR during Veh administration compared to the sham animals; nevertheless CNO administration reduced HHVR. *B*, summary R_f data in NN and HH during Veh and CNO administration in sham (n = 4) (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 6) = 66, P < 0.001) and DREADD's(Gi) animals (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 14) = 236, P < 0.001). *C*, neither groups (sham nor DREADD(Gi)(n = 8)) increased V_t in HH (sham: repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 6) = 0.4, P < 0.55); DREADD's(Gi): repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 7) = 4, P < 0.08); however CNO administration reduced V_t significantly compared to the Veh condition in DREADD's(Gi) animals. *D*, sham animals displayed comparable $\dot{V}_E$ increase in HH during Veh and CNO (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 6)= 47, P < 0.001). However although DREADD's(Gi) animals showed a similar ventilatory response in HH during Veh, CNO blunted the $\dot{V}_E$ response to HH. In HH the $\dot{V}_E$ response during CNO was significantly reduced compared to the Veh condition in DREADD(Gi) rats (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DFn = 1, DFd = 7) = 5, P < 0.05). The normality assumption was performed with the Shapiro-Wilk test, and the W- and *P*-values for every dataset are shown on the left side of every bar.

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Table 2. Respiratory response during NN and HH in sham and DREADD(Gi) animals during Veh and CNO administration in non-anaesthetized preparation

	Sham ($n = 4$)				DREADD(Gi) ($n = 8$)			
	Veh		CNO (1 mg/kg)		Veh		CNO (1 mg/kg)	
	NN	HH	NN	HH	NN	HH	NN	HH
V_t (ml/100 g)	0.61 ± 0.1	0.67 ± 0.3	0.57 ± 0.2	0.59 ± 0.1	0.73 ± 0.5	0.79 ± 0.5	0.54 ± 0.2	0.48 ± 0.2
R_f (breaths/min)	104.5 ± 13.5	165.7 ± 13.1 *$P = 0.0009$	89.5 ± 9.0	143.7 ± 17.5 *$P = 0.0017$	106.7 ± 13.2	157.2 ± 13.8 *$P < 0.0001$	119.8 ± 22.7	156.7 ± 15.1 *$P < 0.0001$
$\dot{V}_E$ (ml/min/100 g)	64.7 ± 14.1	109.8 ± 38.4 *$P = 0.0023$	51.4 ± 15.6	85.8 ± 19.8 *$P = 0.0091$	76.5 ± 46.9	121.7 ± 78.4 $P =$ *00003	63.0 ± 28.4	73.6 ± 32.3

Note: Data are shown as mean ± SD. The data were analysed using repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*.
Abbreviations: CNO, clozapine-*N*-oxide; DREADD's Gi, designer receptors exclusively activated by designer drugs transfected animals; HH, hypobaric hypoxia (PO_2: 100 mmHg); NN, normobaric normoxia (PO_2: 155 mmHg); R_f: respiratory frequency; $\dot{V}_E$, minute ventilation; Veh, NaCl 0.9%; V_t: tidal volume.
*significant *vs.* NN.

Figure 6. ΔEffects of hypobaric-hypoxic on normobaric normoxia during inhibition of carotid body (CB) glomus cells
A, sham animals ($n = 4$) showed a comparable increase in respiratory frequency (R_f) in hypobaric hypoxia (HH) with and without clozapine-*N*-oxide (CNO); however, DREADD(Gi) animals ($n = 8$) depicted a lesser increment of R_f in HH during CNO than Veh condition (paired *t* test, $t = 3.22$, df = 7). *B*, the Δtidal volume ($\Delta\dot{V}_t$)

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HH-normobaric normoxia (NN) response was significantly reduced during CNO compared to the Veh condition in DREADD's(Gi) rats (paired *t* test, $t = 2.18$, df = 14). (**D$_1$**), the Δminute ventilation ($\Delta\dot{V}_E$) HH-NN response was significantly reduced during CNO compared to the Veh condition in DREADD's(Gi) rats (paired *t* test, $t = 2.77$, df = 14). The normality assumption was performed with the Shapiro-Wilk test, and the W- and *P*-values for every dataset are shown on the left side of every bar.

Fig. 6*C*). Together these results indicate that CB glomus cells contribute to the ventilatory chemoreflex response to hypoxia, and that their chemogenetic inhibition dampens this response.

Effect of chemogenetic inhibition of CB glomus cells on lactate-dependent ventilatory and metabolic responses in a non-anaesthetized preparation

In a separate series we evaluated whether lactate infusion could increase ventilation and oxygen uptake at rest, and whether this response was modulated by CNO administration in DREADD(Gi) animals. Lactate infusion significantly increased ventilation (R_f: +38.5 ± 46.4 %; $\dot{V}_E$: +38.0 ± 29.4%) compared to baseline (Fig. 7*A*, *B* and *D*), consistent with prior results (Fig. 1). These ventilatory responses were notably reduced by CNO administration (R_f: +12.8 ± 30.4%; $\dot{V}_E$: +5.6 ± 30.9%) (Fig. 7*A*, *B* and *D*). Of note lactate infusion significantly increased $\dot{V}_{O_2}$ (+40.0 ± 29.2%) (Fig. 7*E*) and $\dot{V}_{CO_2}$ (+39.7 ± 17.2%; Table 3), both of which were blunted following CNO administration ($\dot{V}_{O_2}$: +16.7 ± 25.4%; $\dot{V}_{CO_2}$: +16.5 ± 12.9%; Fig. 7*A*, *B*, *D* and *E*; Table 3). No significant differences in V_t (Fig. 7*A* and *C*) or $\Delta\dot{V}_{O_2}$ (Fig. 7*F*) were observed between conditions.

Plausible role for the CB glomus cells in peak oxygen uptake and exercise performance

To evaluate whether CB glomus cells activity contribute to peak oxygen uptake ($\dot{V}_{O_2peak}$), and consequently exercise performance, we compared $\dot{V}_{O_2peak}$ before and after CNO administration during incremental exercise (Fig. 8*A*) in DREADD(Gi) animals. In both conditions exercise led to marked increases in $\dot{V}_{O_2}$ compared to resting values (Veh: 82.1 ± 18.3 *vs.* 32.1 ± 6.2 ml/kg/min; CNO: 45.9 ± 17.9 *vs.* 21.2 ± 15.9 ml/kg/min; Fig. 8*B*). However $\dot{V}_{O_2peak}$ was significantly reduced under CNO compared to vehicle (45.9 ± 17.9 *vs.* 82.1 ± 18.3 ml/kg/min, Fig. 8*B*), indicating impaired maximal oxygen uptake following CB inhibition. Despite this reduction maximal aerobic speed (i.e. treadmill speed at $\dot{V}_{O_2peak}$) was similar between conditions (Fig. 8*D*).

Discussion

The primary objective of this study was to determine the role of the CB in regulating peak oxygen consumption during physical exertion in rats, following acute chemo-

Table 3. Respiratory and metabolic response to lactate in DREADD(Gi) animals during Veh and CNO administration in non-anaesthetized preparation

	DREADD's Gi (*n* = 4)			
	Veh		CNO (1 mg/kg)	
	Baseline	Lactate 1.2 M	Baseline	Lactate 1.2 M
V_t (ml/100 g)	0.67 ± 0.01	0.68 ± 0.04	0.71 ± 0.06	0.70 ± 0.05
R_f (breaths/min)	114.7 ± 10.6	159.0 ± 53.2 *P = 0.044	99.3 ± 24.5	112.0 ± 30.2
$\dot{V}_E$ (ml/min/100 g)	77.6 ± 7.5	107.4 ± 30.2 *P = 0.006	70.3 ± 17.8	77.8 ± 19.2
$\dot{V}_{O_2}$ (ml/min/kg)	34.5 ± 4.9	48.3 ± 12.0 *P = 0.002	35.9 ± 11.9	41.9 ± 15.6
$\dot{V}_{CO_2}$ (ml/min/kg)	34.7 ± 4.8	48.4 ± 10.1 *P = 0.004	35.6 ± 11.9	42.8 ± 9.1 *P = 0.050
RER ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)	1.01 ± 0.01	1.00 ± 0.01	0.99 ± 0.00	1.02 ± 0.03

Note: Data are shown as mean ± SD. The data were analysed using repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*.

Abbreviations: CNO, clozapine-*N*-oxide; DREADD(Gi), designer receptors exclusively activated by designer drugs transfected animals; RER, respiratory exchange ratio; R_f, respiratory frequency; $\dot{V}_{CO_2}$, CO_2 production; $\dot{V}_E$, minute ventilation; Veh, NaCl 0.9%; $\dot{V}_{O_2}$, O_2 uptake; V_t, tidal volume.

*significant *vs.* baseline.

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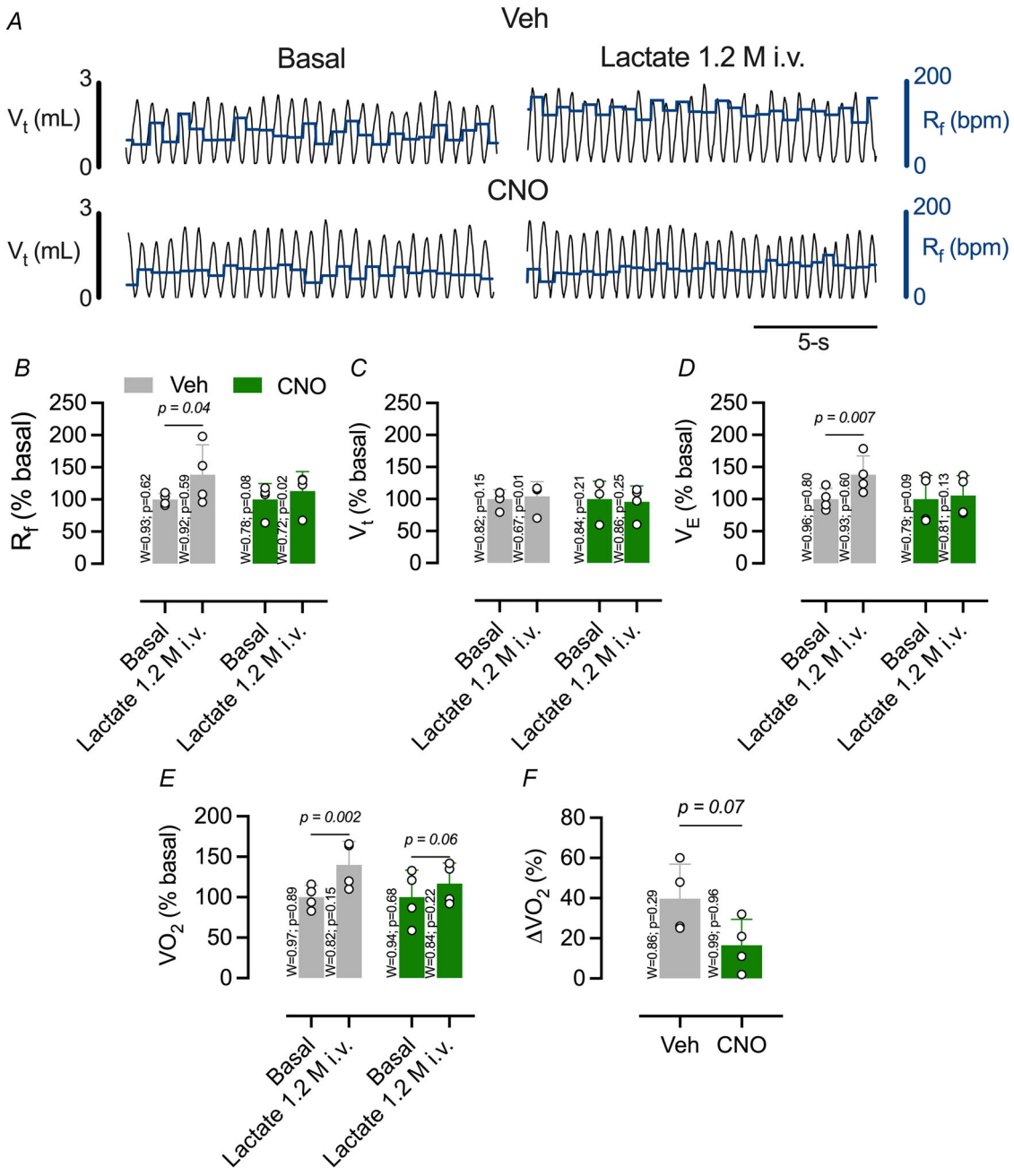


Figure 7. Metabolic and ventilatory responses to lactate during inhibition of carotid body (CB) glomus cells in non-anaesthetized preparation

A, representative ventilatory traces during lactate infusion before (Veh) and after (CNO) silencing CB glomus cells. Notably the animals showed an increase in ventilation with lactate, which was markedly reduced after clozapine-*N*-oxide (CNO, 1 mg/kg, I.P.) administration in DREADD's(Gi) animals. *B*, R_f data at baseline and with lactate infusion during Veh and CNO administration in DREADD(Gi) animals ($n = 4$). The lactate elicited an increment in R_f, which was blunted by CNO administration (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 6) = 6, $P < 0.05$). *C*, lactate did not modify V_t during Veh or CNO in DREADD's(Gi) animals (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 6) < 0.01, $P > 0.99$). *D*, lactate induced an increased ventilation, which was blunted by CNO (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 6) = 11, $P < 0.01$). *E*, percentage increase in O_2 uptake. During the Veh condition $\dot{V}_{O_2}$ was increased to a larger extent than CNO in DREADD's(Gi) animals (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 6) = 29, $P < 0.01$). However the (*F*) $\Delta\dot{V}_{O_2}$ was slightly decreased during CNO (paired *t* test, $t = 2.2$, df = 6). The normality assumption was performed with the Shapiro-Wilk test, and the W- and *P*-values for every dataset are shown on the left side of every bar.

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genetic inhibition of the glomus cells. Our findings support the hypothesis that the CB functions as a lactate sensor (Torres-Torrelo et al., 2021), as evidenced by an arterial-venous lactate clamp, and provide, for the first time, direct evidence that CB glomus cells are crucial for eliciting the metabolic response to maximal physical exercise. Chemogenetic inhibition of these cells was sufficient to reduce NaCN-dependent ventilatory response. Indeed a single small dose of CNO (1 mg/kg, I.P.) reduced CB-dependent ventilatory response to NaCN in awake animals.

Lactate elicits a CB-dependent ventilatory responses *in vivo*

Although isolated CB glomus cells have been shown to sense lactate via Olfr78 (Chang et al., 2015) and/or MCTs (Torres-Torrelo et al., 2021), *in vivo* results have been inconsistent (Peng et al., 2021; Spiller et al., 2021). These discrepancies may stem from interspecies differences (mice *vs.* rats) and lactate delivery method (i.e. bolus *vs.* infusion). Furthermore because lactate exhibits a high metabolic clearance rate, replicating physiological arterial concentrations is critical (Emhoff & Messonnier, 2023;

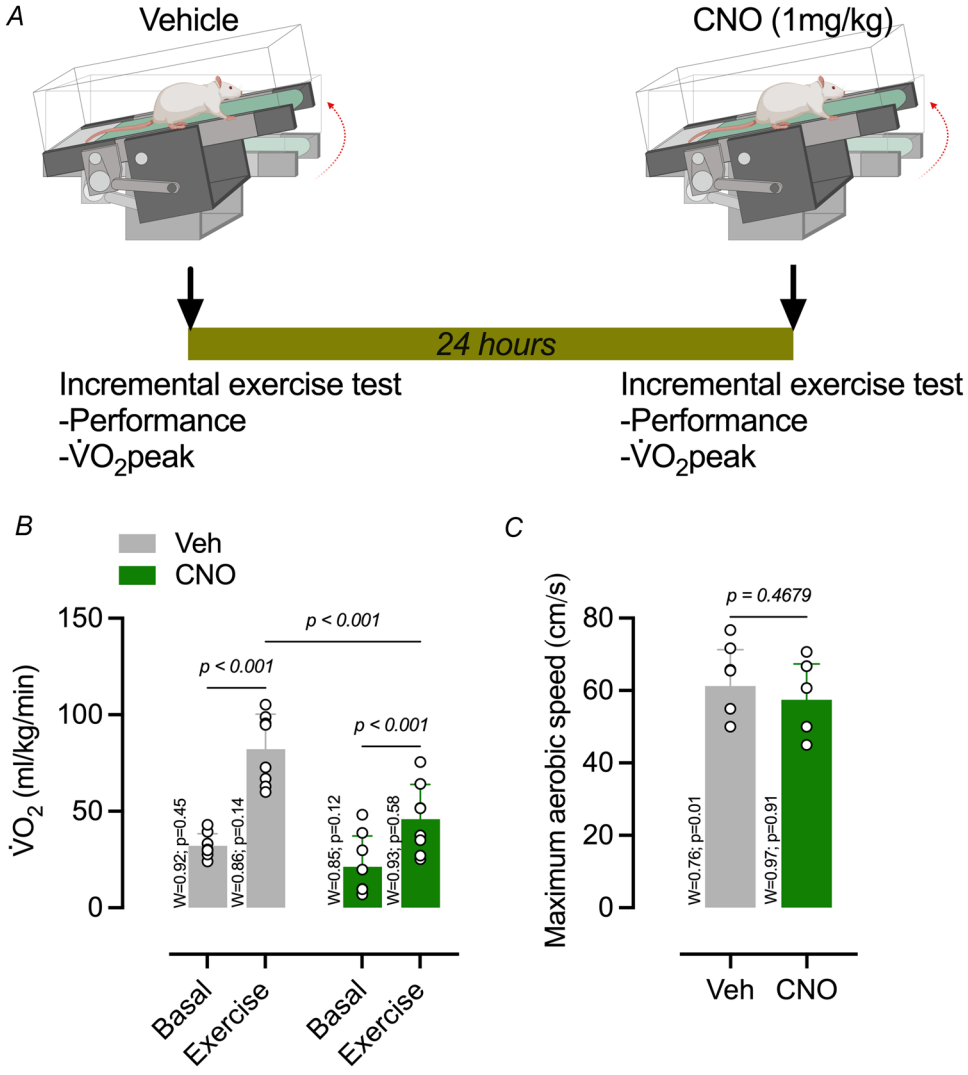


Figure 8. Peak O_2 uptake ($\dot{V}_{O_2peak}$) during physical exertion in DREADD(Gi) animals
A, schematic representation of experimental design regarding $\dot{V}_{O_2peak}$ during vehicle (Veh) and clozapine-*N*-oxide (CNO, 1 mg/kg, I.P.) administration. The same animals underwent two incremental exercise tests to determine $\dot{V}_{O_2peak}$ and maximum aerobic speed, first during Veh and after 24 h during CNO. *B*, both Veh and CNO conditions induced an increase in $\dot{V}_{O_2peak}$, without differences at baseline; however $\dot{V}_{O_2peak}$ was increased to a larger extent during CNO than Veh condition (repeated-measures ANOVA (2 × 2) followed by Holm-Sidak *post hoc*, F(DF*n* = 1, DFd = 14) = 82, $P < 0.001$). *C*, the maximum aerobic speed was similar between the two conditions (paired *t* test, $t = 0.75$, df = 14). $N = 8$. The normality assumption was performed with the Shapiro-Wilk test, and the W- and *P*-values for every dataset are shown on the left side of every bar.

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Juel, 1997). To uncover the mechanisms underlaying how CB senses lactate *in vivo* we performed an arterial-venous lactate clamp to fix arterial lactate concentration during venous infusion, before and after CBR. We demonstrated that arterial lactate was sufficient to induce increased ventilation, which was reduced after CBR. Importantly although we infused 1.2 M lactate venously, the resulting arterial concentration (∼16 mM) was comparable to levels observed during physical exertion in both humans (Hofmann & Tschakert, 2017) and rats (Tsumiyama et al., 2014).

Some studies have reported lower lactate level (e.g. ∼5 mM) at exhaustion (Spiller et al., 2020), suggesting our concentration may be supraphysiological. However other reports showed that lactate can reach ∼13–16 mM during maximal cardiopulmonary exercise test in athletes (Astrand et al., 1960; Cerda-Kohler et al., 2016; Michaelides & Parpa, 2025). Along with previous studies Pilis et al. (1993) demonstrated that the lactate concentration was ∼16 mM in Wistar rats exercising at a similar speed to the one currently use (Pilis et al., 1993). Therefore our data are consistent with previous human and animal data, showing that during high-intensity exercise there is a massive release and accumulation of lactate sufficient to evoke carotid body activation (10–20 mM (Chang et al., 2015; Torres-Torrelo et al., 2021) and, consequently, an increase in ventilation. Thus considering the previous evidence our arterial-venous lactate clamp experiment accumulated a relevant physiological concentration of lactate (∼16 mM) rather than a supraphysiological concentration.

Our results suggest that CB glomus cells detect metabolic stress, likely through lactate accumulation, and promote increased minute ventilation. These results align with previous studies showing lactate sensing via Olfr78 (Chang et al., 2015) and/or MCT in isolated CB glomus cells (Torres-Torrelo et al., 2021). Although Spiller et al. (2021) found no ventilatory response to 20 mM lactate in hemisected Wistar rats, they did not measure arterial lactate concentrations during infusion. In contrast Olsson et al. (2002) showed that 2 mM lactate elicited strong breathing response in female and male Wistar rats.

Another possible explanation for our results is that lactate is released within the CB itself, either by the glomus cells or the sustentacular cells. However Torres-Torrelo et al. (2021) ruled out this mechanism, showing that direct application of pyruvate (a lactate precursor) to the CB glomus cells did not activate them. Instead they demonstrated that lactate-dependent NADH and reactive oxygen species production inhibits nearby membrane voltage-gated K^+ channels, leading to glomus cell depolarization.

It is worth noting that arterial lactate was measured in the femoral region, not near the carotid artery bifurcation – potentially a source of bias. Nevertheless the short half-life of lactate in blood following high-intensity exercise (∼20–35 min; Almenoff et al., 1989; Kappenstein et al., 2015), along with the saturation of MCTs at the clamped concentration used (∼16 mM) (Hertz & Dienel, 2005), suggests that lactate levels measured in the femoral region closely reflect those at the carotid artery bifurcation. These results support that the CB detects lactate *in vivo* and increases ventilation at rest.

Chemogenetic CB glomus cell inhibition reduces NaCN-induced ventilatory response

It has been proposed that the CBs are required to trigger a metabolic response during physical exertion (Andrade, 2024; Honda et al., 1979; Stickland et al., 2007, 2008, 2011), although definite proof remained elusive (Welch & Mitchell, 2024). To address this we first used chemogenetic silencing of CB glomus cells to investigate their role in mediating NaCN-dependent CSN afferent discharges and ventilatory responses. NaCN has long been used as a classical stimulus to activate the CB and assess its function (Iturriaga et al., 2021; Pijacka et al., 2016), including the induction of hyperventilation (Del Rio et al., 2013, 2016). Notably the NaCN-dependent ventilatory response is markedly reduced following CB denervation in animal models of heart failure and hypertension (Del Rio et al., 2013, 2016).

Our results showed that a single dose of CNO was sufficient to inhibit CB glomus cell activity and reduce both the CSN chemosensory activation and the ventilatory response to NaCN. This confirms that our chemogenetic approach effectively attenuates both afferent and efferent components of the chemoreflex. In addition we observed that NaCN tended to increase BP and HR in both vehicle and DREADD(Gi) animals, but these effects were blunted by CNO only in transfected rats. Interestingly we also observed a trend towards decreased BP at baseline during CNO administration in transfected animals, suggesting potential off-target cardiovascular effects (Gomez et al., 2017; Martinez et al., 2019). Although we detected only mild, non-significant effects of CNO on resting CSN activity in DREADD(Gi) rats (non-significant data not shown), the slight reduction in baseline CSN signalling may contribute to the observed decrease in BP.

Together these data demonstrate that chemogenetic silencing of CB glomus cells is sufficient to reduce the NaCN-dependent cardiorespiratory response in DREADD(Gi) animals, mirroring the effects observed following CB denervation in hypertension and heart failure models (Del Rio et al., 2013, 2016). This supports the conclusion that the primary drivers of these effects are the glomus cells themselves.

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Effect of chemogenetic inhibition of the CB glomus cells on hypobaric-hypoxic ventilatory response

The hypoxic ventilatory response is critically dependent on CB integrity in both animals and humans, as demonstrated by reduced responses after bilateral CBR or denervation (Andrade et al., 2021b; Del Rio et al., 2013, 2016; Niewinski et al., 2017). Unilateral CBR does not appear to sufficiently impair the hypoxic ventilatory response in hypertensive patients, indicating that the remaining CB can partially compensate (Narkiewicz et al., 2016).

Our findings align with this previous literature (Andrade et al., 2021b; Del Rio et al., 2013, 2016; Niewinski et al., 2017) and show that chemogenetic inhibition of CB glomus cells effectively reduces the HHVR in non-anaesthetized rats. Interestingly we also observed reduced baseline ventilation in both sham and DREADD(Gi) animals during CNO administration, raising the possibility of CNO-related off-target effects (Gomez et al., 2017; Martinez et al., 2019). However prior studies have shown that only higher doses of CNO (e.g. 10 mg/kg) depress respiration (Martinez et al., 2019), far exceeding the 1 mg/kg dose used in our study. It is worth noting that intermediate CNO doses (e.g. 5 mg/kg) can reduce cocaine-induced locomotion (Padovan-Hernandez & Knackstedt, 2018), and recent work has linked increased breathing during locomotion to the mesencephalic locomotor region and spinal cord through the activation of the pre-Bötzinger complex and central chemoreceptors (Hérent et al., 2023). Therefore reduced baseline ventilation may reflect an indirect behavioural effect of CNO rather than a direct action on the ventilatory control network.

Although CNO can alter behaviour at high doses, such effects can be minimized by habituating the animal to handling and injection (Martinez et al., 2019). Nevertheless further studies are warranted to thoroughly evaluate CNO off-target effects on ventilatory circuits and metabolic regulation, especially across different species and rat strains (e.g. Sprague–Dawley vs. Wistar Kyoto).

Effect of chemogenetic inhibition of CB glomus cells on lactate-dependent ventilatory and metabolic responses in non-anaesthetized rats

Two primary mechanisms have been proposed for lactate sensing by the CB: one involving the olfactory receptor Olfr78 (Chang et al., 2015; Peng et al., 2020, 2021) and another involving MCTs (Torres-Torrelo et al., 2018, 2021). In the Olfr78 pathway lactate binding leads to increased adenylyl cyclase activity, elevated cAMP, intracellular calcium influx and the and release of excitatory neurotransmitters from CB glomus cells, ultimately stimulating ventilation (Peng et al., 2023). However recent evidence from TH-Olfr78$^{-/-}$ mice suggest that this receptor may not be essential for eliciting ventilatory responses to hypoxia (Colinas et al., 2024). The MCT-dependent mechanism proposes that lactate enters glomus cells via MCTs, is converted to pyruvate by lactate dehydrogenase and produces NADH. This NADH may then activate membrane cation channels, causing depolarization and neurotransmitter release (Torres-Torrelo et al., 2021). Another possible mechanism could be related to the intracellular acidification: elevated $[H^+]_i$ inhibits TASK1 channels, leading to depolarization, calcium influx, transmitter release and CSN activation (Iturriaga et al., 2021). Although we did not directly assess intracellular pH in our experiments, this mechanism remains plausible.

Our results provide *in vivo* evidence that lactate not only increases ventilation but also enhances $\dot{V}_{O_2}$, supporting the role of the CB as a metabolic sensor. Previous studies in hemisected Wistar rats or isolated glomus cells have reported blunted ventilatory responses to lactate (Spiller et al., 2021), though these studies did not verify arterial lactate levels. In contrast (Olsson et al., 2002) showed robust ventilatory responses to 2 mM lactate infusions in both female and male Wistar rats. Given the high metabolic clearance rate of lactate it remains unclear whether CB glomus cells respond to absolute lactate concentration or dynamic changes (Emhoff & Messonnier, 2023; Juel, 1997).

CB glomus cells' role in peak O_2 uptake and exercise performance

After confirming that DREADD(Gi)-mediated activation of CB glomus cells blunts both chemosensitivity and lactate-dependent increases in $\dot{V}_{O_2}$ and ventilation, we assessed whether these cells are necessary to elicit metabolic responses during physical exertion. Following CNO administration we observed reduced lactate-dependent $\dot{V}_{O_2}$ and ventilation at rest, as well as a marked reduction in $\dot{V}_{O_2peak}$ during maximal exercise. This strongly supports the notion that CB glomus cells are required to reach $\dot{V}_{O_2peak}$ during high-intensity exercise.

Interestingly despite the reduction in $\dot{V}_{O_2peak}$, exercise performance was not affected, suggesting that CB glomus cell inhibition may improve metabolic efficiency, achieving the same maximum aerobic speed with a lower oxygen cost. These findings, although novel, require further confirmation. To our knowledge this is the first direct demonstration of CB glomus cells as key contributors to the metabolic response during exercise.

Early evidence of CB involvement in exercise metabolism comes from studies showing that hyperoxic gas administration at the second ventilatory threshold

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reduced pulmonary ventilation during exercise (Masuda et al., 1988). Although hyperoxia has confounding effects, such as acidosis, and altered norepinephrine release (Dempsey & Smith, 1994), it is a well-known method to transiently silence CB activity (Dejours, 1962). Moreover CBR in asthma patients was shown to attenuate $\dot{V}_{O_2}$ kinetics and respiratory responses in all phases of a CPET (Honda et al., 1979). Similarly we previously reported that CBR severely impaired running capacity in rats with heart failure (Andrade et al., 2021a).

Our current findings are consistent with these earlier results and extend them by specifically highlighting the metabolic, rather than performance-related, consequences of CB glomus cell inhibition. Conflicting results in ponies showed enhanced hyperventilation after CB denervation (Pan et al., 1986), but these animals also exhibited CO_2 retention at rest, which may sensitize central chemoreceptors and amplify ventilatory responses during exercise. In contrast CO_2 retention has not been reported in humans (Honda et al., 1979; Masuda et al., 1988), and in our experiments CO_2 production remained unchanged during CNO administration in DREADD(Gi) animals.

In summary we provide the first evidence that CB glomus cells act as sensors of metabolic status and facilitators of the metabolic response to maximal exercise. Our findings support that exercise-induced lactate accumulation activates the CB, thereby enhancing ventilation and oxygen uptake through a positive feedback loop.

Limitations

This study is not free of limitations. First we did not identify the molecular entities related to lactate sensing during exercise, and how it is activated in the CB glomus cells. However our goal was to determine the physiological contribution of CB glomus cells to sensing and triggering a metabolic response to physical exercise, rather than to pinpoint the receptor(s) involved. Second we employed anaesthetized preparations for the arterial-venous lactate clamp and for validating chemogenetic silencing using NaCN, which could be seen as a limitation. Nevertheless all animals underwent identical procedures, mitigating the impact of anaesthesia-related bias. Importantly we replicated the ventilatory effects of lactate in non-anaesthetized animals as well. Third although we clamped arterial lactate at ∼16 mM, this concentration could be considered supra-physiological. However lower lactate levels (∼4 to 5 mM) reported during exercise exhaustion (Spiller et al., 2020) were obtained using protocols with longer stages and lower intensity, potentially limiting lactate release and accumulation. Moreover 4–5 mM lactate is closer to the lactate threshold than the maximum lactate accumulation, whether running (Pilis et al., 1993) or swimming (Voltarelli et al., 2002). In contrast our infusion protocol more accurately mimics high-intensity exercise. Nonetheless we did not report arterial lactate concentrations in the non-anaesthetized condition, which is a significant limitation. Additionally we did not include experiments with DREADD(Gq), which would have been a valuable complementary approach to explore whether CB activation enhances the metabolic response and exercise performance, potentially acting as an ergogenic agent.

Conclusions

This study demonstrates that CB glomus cells are essential for mounting an appropriate metabolic response during high-intensity exercise, inducing a lower $\dot{V}_{O_2peak}$. Moreover our findings support the role of lactate as a key physiological signal for CB activation at exertion. These results provide new insights into the carotid body's function as a metabolic sensor and open avenues for further research on its role in exercise physiology.

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Additional information

Data availability statement

The datasets are available from the corresponding authors upon request.

Competing interests

None of the authors declare competing financial interests.

Author contributions

D.C.A.: writing of the original draft, review and editing, project administration, resources, methodology, investigation and conceptualization. C.S.-A.: participates in the original draft, formal analysis, data curation and review and editing. C.T.: formal analysis, data curation and review and editing. C.A.: writing, review and editing. E.D.-J.: formal analysis, data curation, writing, review and editing. R.I.: formal analysis, data curation, writing, review and editing. M.R.-F.: writing, review and editing. G.P.M.: writing, review and editing. All authors approved the final version of the manuscript.

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carotid body, exercise, hypoxia, lactate, maximal oxygen uptake, peripheral oxygen chemoreflex, $\dot{V}_{O_2peak}$

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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