Lactate sensing mechanisms in arterial chemoreceptor cells

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Classically considered a by-product of anaerobic metabolism, lactate is now viewed as a fundamental fuel for oxidative phosphorylation in mitochondria, and preferred over glucose by many tissues. Lactate is also a signaling molecule of increasing medical relevance. Lactate levels in the blood can increase in both normal and pathophysiological conditions (e.g., hypoxia, physical exercise, or sepsis), however the manner by which these changes are sensed and induce adaptive responses is unknown. Here we show that the carotid body (CB) is essential for lactate homeostasis and that CB glomus cells, the main oxygen sensing arterial chemoreceptors, are also lactate sensors. Lactate is transported into glomus cells, leading to a rapid increase in the cytosolic NADH/NAD$^+$ ratio. This in turn activates membrane cation channels, leading to cell depolarization, action potential firing, and Ca$^{2+}$ influx. Lactate also decreases intracellular pH and increases mitochondrial reactive oxygen species production, which further activates glomus cells. Lactate and hypoxia, although sensed by separate mechanisms, share the same final signaling pathway and jointly activate glomus cells to potentiate compensatory cardiorespiratory reflexes.
While lactate has classically been considered a waste product of anaerobic metabolism and a fatigue agent, this view has changed radically in recent years. It is now established that even under fully aerobic conditions, lactate is the final product of glycolysis in many cell types. Lactate can be secreted, for example, by white skeletal muscle, erythrocytes or astrocytes and either consumed by neighboring cells (e.g., neurons) or transported by the blood to cells in distant organs for energy production (e.g., in the heart) or gluconeogenesis (e.g., in the liver). In lactate-consuming cells, lactate is converted to pyruvate by lactate dehydrogenase in the cytosol or in the mitochondrial intermembrane space. In addition to being a systemic metabolic substrate, preferred over glucose as a fuel in numerous tissues, lactate is gaining relevance for its role as a signaling molecule. Lactate is co-transported with protons into cells by a broadly distributed family of reversible monocarboxylate transporters (MCTs). It can also bind to GPR81, a G-protein coupled putative lactate receptor expressed in adipose tissue and brain. Lactate inhibits lipolysis, favors angiogenesis through the induction of VEGF, and elicits vasodilation. Infused lactate increases circulating BDNF levels, protects heart and ischemic neurons, promotes adult hippocampal neurogenesis, and inhibits inflammation following organ injury.

Moreover, lactate’s interaction with histidine residues activates TREK1 K+ channels into cells by a broadly distributed family of reversible monocarboxylate transporters (MCTs). It can also bind to GPR81, a G-protein coupled putative lactate receptor expressed in adipose tissue and brain. Lactate inhibits lipolysis, favors angiogenesis through the induction of VEGF, and elicits vasodilation. Infused lactate increases circulating BDNF levels, protects heart and ischemic neurons, promotes adult hippocampal neurogenesis, and inhibits inflammation following organ injury.

Despite the vast amount of existing literature on lactate metabolism and signaling in numerous organs, the “where” and “how” blood lactate (lactatemia) is sensed to elicit adaptive responses in the organism are unknown. Acute hyperlactatemia occurs in response to physical exercise or exposure to systemic hypoxia, but the homeostatic role of these responses is not well understood. It has been suggested that lactate itself, apart from lactic acidosis, can induce ventilation and contribute to the initiation and maintenance of the hypoxic ventilatory response (HVR), possibly via an action on the carotid body (CB), the main acute O2-sensing organ in mammals. It has also been reported that lactate activates the CB by means of binding to an atypical olfactory receptor (Olfr78) that is highly expressed in the CB’s chemoreceptor (or glomus) cells. This latter proposal was disregarded because the findings could not be reproduced by others. Nonetheless, in preliminary studies, we found that single Olfr78-deficient glomus cells are indeed directly activated by lactate. Here we report that the CB is essential for lactate homeostasis and that glomus cells are physiologically relevant acute lactate sensors. Lactate is transported into glomus cells where it increases the cytosolic NADH/NAD+ ratio, decreases cytosolic pH, and stimulates mitochondrial production of reactive oxygen species (ROS), which in turn activate cation channels to induce membrane depolarization, Ca2+ entry, and transmitter release. Lactate and hypoxia, although sensed by separate mechanisms, jointly activate glomus cells to potentiate adaptive cardiorespiratory reflexes.

Results and discussion

**Carotid body dysfunction enhances hypoxia-induced lactatemia.** The main systemic acute O2-sensing organ in mammals is the CB, which upon activation by hypoxia stimulates the central respiratory and autonomic centers to induce hyperventilation and sympathetic activation. We tested whether the CB plays any role in lactate homeostasis by monitoring hypoxia-induced lactatemia in wild type mice (control) compared to mice insensitive to hypoxia due to deficient CB function. To this end, we used two mouse models previously studied in our laboratory: TH-HIF2a, with embryonic ablation of the gene Epas1 (coding HIF2α) in sympatheticadrenal (tyrosine hydroxylase-positive) cells and exhibiting CB atrophy and strong inhibition of the HVR (Supplementary Fig. 1), and ERT2-HIF2a, with ubiquitous conditional ablation of Epas1 and showing normal CB development but selective abolition of responsiveness to hypoxia in adulthood. In wild type mice, hypoxia, even if relatively mild, induced a graded and reversible increase in blood lactate within a few minutes that occurred in parallel with an increase in ventilation (Fig. 1a and c). In contrast, hypercapnia (5% CO2), a stimulus known to activate CB cells, had no effect on blood lactate but did evoke a ventilatory response stronger than that induced by hypoxia (Fig. 1b and c). Lactatemia elicited by breathing 10% O2, was much higher in CB-deficient mice (~30% and ~90% increase in TH-HIF2a and ERT2-HIF2a strains, respectively) than in controls (Fig. 1d).

These data indicate that although hypercapnia induces, as hypoxia, CB activation and hyperventilation it is not accompanied by lactatemia. CB activation during systemic hypoxia in normal mice reduces the intensity of hypoxemia and thereby blunts lactate release from tissues.

**Ca2+-dependent activation of carotid body glomus cells by lactate.** We studied the effect of extracellular L-lactate (sodium salt) on mouse single glomus cells, the polymodal acute O2-sensing sensory neuronal elements in the CB (Fig. 2a). Amperometric experiments showed that lactate induced a dose-dependent exocytotic catecholamine release from cells in CB slices (Fig. 2b and c). Cytosolic Ca2+ measured in Fura 2-loaded dispersed glomus cells increased in response to high K+ and hypoxia. In ~90% of these O2-sensitive cells, lactate also elicited an increase in cytosolic Ca2+ (Fig. 2d) which was abolished by extracellular cadmium (Cd2+), a non-selective blocker of high-threshold membrane Ca2+ channels in glomus cells (Fig. 2g). Lactate-induced secretory activity was also blocked by Cd2+ (Fig. 2h) and strongly inhibited by nifedipine (Fig. 2i and j), a blocker of L-type Ca2+ channels in rodent glomus cells. Therefore, similar to hypoxia, lactate activates glomus cells in an external Ca2+-dependent manner. In agreement with these data in mouse CB, we recorded robust responses to lactate in dispersed rat glomus cells (Supplementary Fig. 2a) as well as in cells in rat CB slices (Supplementary Fig. 2b).

It has recently been reported that membrane potential and conductance of rat glomus cells are unaffected by lactate, however, these experiments were performed with the whole-cell configuration of the patch-clamp technique and therefore intracellular dialysis may have altered the lactate signaling pathway (see below).

**Lactate is transported into glomus cells and leads to NADH accumulation and mitochondrial ROS production.** In many cell types, lactate is co-transported with H+ across the plasma membrane by monocarboxylate transporters (MCTs) and once inside the cell is converted to pyruvate (in the cytosol or organelles) by lactate dehydrogenase with production of NADH (see scheme in Fig. 3a). We studied by immunocytochemistry the expression in CB cells of MCT1, 2, and 4, the most representative MCTs in mammalian cells. MCT2, a high-affinity (Km < 1 mM) lactate transporter expressed in neurons and other cell types, was consistently detected in tyrosine hydroxylase (TH) positive glomus cells (Fig. 3b), however, this transporter was absent in glia-like, fibrillary acidic protein (GFAP) positive, CB type II cells (Fig. 3c; Supplementary Fig. 3). MCT2 was also expressed in neurons of the superior cervical ganglion (SCG) and in adrenal medulla (AM) chromaffin cells (Supplementary Fig. 4a and b), which are catecholaminergic (TH positive) cells that, as the CB glomus cells, derive from neural crest progenitors. Neither CB glomus cells (Fig. 3d) nor type II cells (Supplementary Fig. 5a and c) expressed MCT1, a ubiquitous lactate transporter with lower affinity (Km ~5 mM) than MCT2.
**Fig. 1 Blunting of hypoxia-induced lactatemia by arterial chemoreceptors.** a, b Top. Scheme of the experimental protocol followed to measure blood lactate (lactate sampling indicated by horizontal brown bars) under normoxic conditions (21% O2) and during exposure to hypoxia (10% or 15% O2 tension, pale blue) or hypercapnia (5% CO2, pale red). Bottom. Plethysmographic recordings showing the average increase in respiratory frequency (breaths/min) during exposure to hypoxia (blue dots; n = 35 mice) or hypercapnia (red dots; n = 27 mice). c Scatter plots of blood lactate levels measured in the various experimental conditions. Values (mean ± SEM) are: normoxia (2.5 ± 0.1 mM, n = 29 mice; gray), hypoxia 15% O2 (3.6 ± 0.3 mM, n = 17 mice; blue), hypoxia 10% O2 (5.8 ± 0.5 mM, n = 23 mice; blue), recovery in normoxia (21% O2 measured 5 min after returning to normoxia; 4.4 ± 0.3 mM, n = 14 mice; gray), and hypercapnia (2.2 ± 0.5 mM, n = 4 mice; pale red). P-values calculated by one-way ANOVA followed by Tukey’s multiple comparisons post hoc test are indicated. d Scatter plots of blood lactate levels, during normoxia (gray) and hypoxia (blue), in control and mice with carotid body dysfunction due to embryonic or conditional adult deficiency of Hif2α. P-values calculated by one-way ANOVA followed by Tukey’s multiple comparisons post hoc test are indicated. Mean ± SEM values are: TH-HIF2α (normoxia = 3.2 ± 0.6 mM; hypoxia 10% O2 = 8.3 ± 1.2 mM, n = 5 mice); ERT2-HIF2α (normoxia 2.9 ± 0.4 mM; hypoxia 10% O2 = 12.3 ± 1.7 mM, n = 4 mice). Source data are provided as a Source data file.

However, TH positive CB cells expressed MCT4 (Fig. 3e), which was considered to be a low affinity (Km > 20 mM) transporter characteristic of lactate secreting cells38,40. A recent study indicates that MCT4 affinity for lactate (Km ~1 mM) is much higher than previously thought and that this transporter has a relevant affinity for pyruvate (Km ~4 mM). However, the higher affinity for lactate relative to pyruvate makes MCT4 suitable for a major role in lactate release from cells41. MCT4 appeared to be only slightly expressed in some type II cells (Supplementary Fig. 5b and d). The selective abundant expression of MCT2 in glomus cells suggest that they can easily uptake lactate from blood, a property compatible with their role as lactate sensors. MCT4 may also contribute to lactate uptake by glomus cells. This transporter, which is less sensitive to pyruvate than to lactate38,41, could also mediate lactate secretion from glomus cells in some circumstances, as for example in conditions of accelerated glycolysis secondary to mitochondrial dysfunction42. MCT4 might also be preferentially expressed in CB neuroblasts, which are immature TH-positive CB cells with the mitochondria-based acute O2-sensor still undeveloped43. Noteworthy, type II cells, which are glia-like supportive elements with quiescent stem cell function44,45, did not express MCT1 and seemed to have only low levels of MCT4 expression. In this regard, type II cells are different from astrocytes which release lactate to fuel and modulate the function of neighboring neurons3,39,46. In agreement with the immunocytochemical observations, we monitored lactate transport into glomus cells by measuring intracellular acidification using single-cell microfluorimetry (Fig. 3f and g). Control of the lactate-induced acidification signal was obtained by exposure of the cells to CO2, which strongly acidifies the cytosol due to its conversion to carbonic acid by carbonic anhydrase present in CB glomus cells47.
Exposure of dispersed glomus cells to lactate elicited a fast, highly reversible, and dose-dependent increase in intracellular NADH, as monitored by single-cell NAD(P)H autofluorescence. This signal was strongly inhibited by AR-C155858 (Fig. 4c and d), an inhibitor of MCT1 and MCT2. The lactate (10 mM)-dependent increase in NADH was abolished by the extracellular application of pyruvate (5 mM), a molecule also co-transported with H⁺ by MCT1 and MCT2 but with a higher affinity (Km < 1 mM and <0.1 mM for MCT1 and MCT2, respectively) than lactate. Application of a short pulse of pyruvate produced a marked decrease in the lactate-induced NADH signal to values below even to basal levels, indicating that once inside the glomus cell, pyruvate, by consuming NADH, was rapidly converted to lactate by lactate dehydrogenase. Treatment with α-ketobutyrate (α-KB), which was shown previously to rapidly decrease NADH autofluorescence in glomus cells due to its conversion to non-metabolizable α-hydroxybutyrate, also prevented the lactate-dependent NADH rise in the cells.

In addition to decreasing NADH levels, pyruvate inhibited the secretory response to lactate in glomus cells (Fig. 4g). Even a small amount of extracellular pyruvate (100 μM), near the normal concentration in plasma, was sufficient to partially inhibit lactate-induced secretion in glomus cells (Supplementary Fig. 6c and d). However, normal responses to high lactate were recorded in cells bathed by solutions containing physiological plasma levels of pyruvate and lactate. Although the presence of lactate a short exposure to pyruvate consistently inhibited the secretory activity in glomus cells, in most cases this inhibitory phase was followed by a late activation of the cells (Supplementary Fig. 6e). Indeed, although at high non-physiological concentrations, pyruvate by itself (in the absence of lactate) induced a slow secretory activity in all cells tested probably due to intracellular acidification (Supplementary Fig. 6f). These results suggest that increases in extracellular lactate alter the extra and intracellular pyruvate/lactate equilibria, resulting in NADH accumulation (or an increase in the NADH/NAD⁺ ratio), which participates in the regulation of Ca²⁺ influx by lactate in
these cells (see below). In addition, intracellular acidification produced by lactate transport may also contribute to activation of glomus cells. Together with NADH measurements we monitored the generation of mitochondrial ROS in glomus cells using a fluorescent genetic probe selectively directed to the mitochondrial matrix or intermembrane space (IMS)\(^\text{30}\). Lactate produced a dose-dependent reversible rise in matrix ROS (Fig. 4i, top), which likely reflected the increased fuel supply to the highly active glomus cell mitochondria\(^\text{30,52}\). Indeed, pyruvate at the same concentration also produced an increase in matrix ROS, which produced by lactate or pyruvate was also manifested in the IMS (Supplementary Fig. 6g and h).

**Lactate-induced depolarization and action potential firing is triggered by NADH-dependent cation channels.** Because lactate induction of secretory activity in glomus cells was dependent on \(\text{Ca}^{2+}\) influx, we hypothesized that lactate might elicit membrane depolarization and, in this way, give rise to the opening of voltage-gated \(\text{Ca}^{2+}\) channels. Experiments on patch-clamped (perforated patch configuration) dispersed glomus cells showed that lactate (10 mM) elicited a reversible depolarization of 9.5 ± 1 mV (\(n = 17\) cells) from a relatively low spontaneous basal resting potential (RP \(\approx -30 \text{ to } -40\) mV) (Fig. 5a and b). When current clamped cells were hyperpolarized to \(-70\) mV to de-inactivate voltage-gated ion channels, lactate evoked a larger (31 ± 4 mV; \(n = 16\) cells) depolarization accompanied by the firing of action potentials (Fig. 5b and c). Because glomus cells have a \(\text{Na}^{+}\)-permeant standing conductance at rest\(^\text{33,34}\), replacement of extracellular \(\text{Na}^{+}\) with N-methyl-D-glucamine (NMDG, a large membrane-impermeant cation) resulted in cell hyperpolarization and abolition of the response to lactate (Fig. 5d). As shown above for the recordings of NADH autofluorescence and glomus cell secretion, application of a fast pulse of pyruvate rapidly and reversibly inhibited the cell depolarization and action potential firing induced by lactate (Fig. 5e).

Finally, the electrophysiological effects of lactate (Fig. 5f) and lactate-induced secretory activity (Fig. 5g) were abolished by 2-APB, a non-selective blocker of some transient receptor potential (TRP) and other cationic channels in CB\(^\text{35}\). Taken together, these data suggest that lactate-induced increase in the cytosolic NADH/NAD\(^{+}\) ratio leads to a cation channel-dependent depolarization of glomus cells with subsequent opening of \(\text{Ca}^{2+}\) channels, \(\text{Ca}^{2+}\) influx, and transmitter release. SCG neurons and AM chromaffin cells showed lactate-induced increases in NAD(P)H autofluorescence (Supplementary Fig. 7a, c, e), which is compatible with the expression of MCT2 in these cells (see Supplementary Fig. 4). However,
patch clamped dispersed SCG neurons that were reversibly depolarized by high extracellular K⁺ were unaltered, or even slightly hyperpolarized, by lactate (Supplementary Fig. 7b, d). On the other hand, hypercapnia induced catecholamine release from adult AM chromaffin cells[^56], however, these cells appeared to be practically insensitive to lactate (Supplementary Fig. 7f). These observations further support a selective role as lactate sensors for CB glomus cells. The differential sensitivity of glomus cells to lactate, respecting SCG neurons or AM chromaffin cells, may be a consequence of the different expression of membrane ion channels. However, it could be also due to the fact that neither SCG neurons nor AM chromaffin cells are able to generate a relevant mitochondrial ROS signal in response to lactate.

Pyridine nucleotides are promiscuous signaling molecules that can regulate, directly or indirectly, a large variety of ion channel families[^57], most of which are represented in glomus cells[^52,58] and therefore could mediate lactate-dependent depolarization. A good candidate forming part of this response is the TRPC5 channel because the mRNA coding this protein is highly abundant in glomus cells[^30] and in a comparative microarray analysis we showed that TRPC5 mRNA is more highly expressed in the CB than in SCG or AM cells[^52]. To test this possibility, we performed experiments on CB glomus cells from TRPC5 families[^57, most of which are represented in glomus cells[^27,30,52,58]. To test this possibility, we performed experiments on CB glomus cells from TRPC5

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Fig. 5 Depolarization and action potential firing induced by lactate in glomus cells. a–c Representative examples of lactate (10 mM)-induced reversible depolarization in a current clamped cell (perforated patch) maintained at the spontaneous resting potential (RP ≈ 30–40 mV). d In hyperpolarized to ~70 mV by current injection (red line and arrow) lactate (10 mM) induced larger depolarizations and the firing of action potentials. b Box plots representing the amplitudes of lactate (10 mM)-induced depolarizations in the two conditions. Mean ± SEM values were: 9.5 ± 1 mV (n = 17 cells from 16 mice) at RP, 31.2 ± 4 mV (n = 16 cells from 10 mice) at ~70 mV. The boxplot represents the median (middle line), 25th, 75th percentile (box), and largest and smallest values extending no further than 1.5× interquartile range (whiskers). Source data are provided as a Source data file. d Cell hyperpolarization and abolition of the lactate (10 mM)-induced depolarization after replacement of extracellular Na+ with N-methyl-D-glucamine (NMDG). Similar results were obtained in all cells tested (7 cells from 4 mice). e Inhibition of lactate (10 mM)-induced depolarization and action potential firing by application of a short pulse of pyruvate (Pyr, 5 mM). Representative example of 8 cells recorded from 5 mice. Red line indicates current injection through the recording electrode to maintain a resting potential of ~70 mV. f Inhibition of lactate (10 mM)-induced depolarization and action potential firing by 2-APB (10 μM), a non-selective cation channel blocker. Representative example of 3 cells recorded from 3 mice. Red line indicates current injection through the recording electrode to maintain a resting potential of approximately ~70 mV. Note that action potential amplitude is truncated due to the relatively long sampling interval (4 ms). g Reversible inhibition of the lactate (20 mM)-induced secretory response by 2-APB (10 μM). Representative example of 4 experiments in 3 mice.

knockout mice, which exhibited normal responses to hypoxia and lactate (Supplementary Fig. 8a–c). We also studied CB glomus cells from mice lacking TRPC6 channels, another member of the canonical subfamily of TRP channels59, which is expressed in glomus cells38 and has been suggested to be essential for hypoxic pulmonary vasoconstriction60. Our data indicated that sensitivity to lactate was not significantly affected in either TRPC6-deficient glomus cells or in cells obtained from Trpc5/Trpc6 double knockout mice (Supplementary Fig. 8d–g). In addition to TRPC5, mRNAs coding for subunits of TRPC3 and TRPM7 channels, as well as a beta subunit of non-voltage sensitive epithelial Na+ channels have been reported to be abundant in glomus cells35. Nonetheless, pharmacological experiments with several specific blockers (Pry3, FTY720, and amiloride for TRPC3, TRPM7, and epithelial Na+ channels, respectively61–63 showed that these channel types are not essential for lactate sensing by glomus cells (Supplementary Fig. 9a–d). These results suggest that the intracellular NADH and ROS produced by lactate are promiscuous signals that probably redundantly modulate several types of cationic channels in glomus cells.

Lactate- and O2-sensing are mediated by separate mechanisms. It is well established that activation of CB glomus cells by hypoxia is a cell autonomous process that can be elicited in the absence of lactate or any other endocrine or paracrine signal27. Recently, it was shown that hypoxia-induced glomus cell transmitter release depends on both NADH, shuttled from the mitochondria to the cytosol, and ROS that are primarily generated at the mitochondrial IMS (probably at mitochondrial complexes—MC-I and III), which rapidly inhibit nearby membrane K+ channels to induce depolarization and Ca2+ influx34,42,50. During more protracted exposures to hypoxia, cationic channels, activated by intracellular Ca2+ or cytosolic diffusion of the signaling molecules, may also...
Compartmentalized steps in acute oxygen and lactate sensing by carotid body glomus cells. The decrease of oxygen (hypoxia) is detected by mitochondria, induce transmitter release. MCT monocarboxylate transporter, LDH lactate dehydrogenase, de oxygen species at the mitochondria and in this manner contribute to the activation of glomus cells. Both hypoxia and lactate increase cytosolic \([\text{Ca}^{2+}]\) on the other hand, lactate is transported into the cells by MCTs and rapidly converted to pyruvate with the production of NADH, which activates membrane cation channels to produce cell depolarization (an effect that may be facilitated by intracellular acidification).

Cells from these mice showed a secretory response to hypoxia. Hypercapnia (10% CO2, pale red) and K+ (40 mM)-induced depolarization. Similar experiments (n = 6) were performed in CBs from 3 mice. None of the cells from these mice showed a secretory response to hypoxia. Secretory responses to lactate (gray) in a hypoxia-insensitive (pale blue) glomus cell dispersed from a Ndufs2-deficient CB. Box plots representing the distribution of changes in NADH signals (\(\Delta\text{NADH}\)) in 22 cells from 3 mice exposed to the two stimuli. In these experiments, all cells responded to lactate (10 mM) but only 40% of the cells showed some responsiveness to hypoxia (O2 tension \(\approx 15\) mm Hg). Mean ± SEM values are: 10 mM lactate: 52.5 ± 4.3; hypoxia: 28.2 ± 3.2. P value calculated by two tails, unpaired t test is indicated. The boxplot represents the median (middle line), 25th, 75th percentile (box), and largest and smallest values extending no further than 1.5 × interquartile range (whiskers). Source data are provided as a Source data file.

Representative amperometric recordings from an Ndufs2-deficient CB slice showing that cells insensitive to hypoxia (pale blue) are activated by lactate (10 mM, gray), hypercapnia (10% CO2, pale red) and K+ (40 mM)-induced depolarization. Similar experiments (n = 6) were performed in CBs from 3 mice. None of the cells from these mice showed a secretory response to hypoxia. Secretory responses to lactate (gray) in a hypoxia-insensitive (pale blue) glomus cell deficient of the \(\text{Epas1}\) (coding Hif2\(\alpha\)) gene. Similar experiments (n = 2) were performed in CBs from two mice. Schematic representation of the major compartmentalized steps in acute oxygen and lactate sensing by carotid body glomus cells. The decrease of oxygen (hypoxia) is detected by mitochondria, giving rise to the production of NADH (N) and reactive oxygen species (R) which inhibit nearby membrane K+ channels to induce depolarization. On the other hand, lactate is transported into the cells by MCTs and rapidly converted to pyruvate with the production of NADH, which activates membrane cation channels to produce cell depolarization (an effect that may be facilitated by intracellular acidification). Pyruvate can also increase the production of reactive oxygen species at the mitochondria and in this manner contribute to the activation of glomus cells. Both lactate and hypoxia increase cytosolic [Ca\(^{2+}\)] and induce transmitter release. MCT monocarboxylate transporter, LDH lactate dehydrogenase, \(\Delta V_m\) membrane depolarization. Arbitrary units (arb. units). Cells from mice deficient of Hif2\(\alpha\), which have a normal MCI but strong inhibition of responsiveness to acute hypoxia due to down-regulation of atypical MCIV mitochondrial subunits\(^34\), also showed robust responses to lactate (Fig. 6f). These data indicate that lactate and oxygen sensing are mediated by separate mechanisms, although they share compartmentalized signals (rise of cytosolic NADH/NAD\(^+\) ratio and ROS) that induce depolarization, Ca\(^{2+}\) influx, and glomus cell transmitter release (Fig. 6g).

In support of this notion, we found that between 65 and 90% of glomus cells that responded to hypoxia also responded to lactate (Fig. 7a–c; see Fig. 2d–f). Moreover, both the NADH signal and the secretory response to hypoxia were strongly potentiated by simultaneous application of extracellular lactate (Fig. 7d–g).

In sum, we show here that the carotid arterial chemoreceptors play a major role in lactate homeostasis, a physiological process of broad functional and medical relevance. CB chemoreceptor cells secondarily contribute to this response\(^64\). Here we have shown that lactate activation of glomus cells depends on similar signals (NADH and ROS), although NADH is produced in the cytosol as a consequence of imported lactate and its rapid conversion to pyruvate, and ROS are primarily generated at the mitochondrial matrix. To further determine whether hypoxia and lactate share similar sensing/signaling mechanisms, we tested the effect of rotenone, a blocker of MCI that induces glomus cell secretion and functional down-regulation of atypical MCIV mitochondrial subunits\(^34\), also showed robust responses to lactate (Fig. 6a).

Moreover, while responsiveness to hypoxia was practically abolished in mice with ablation of the Ndufs2 gene, which codes a 49 KDa protein essential for MCI assembly and function\(^42,50\), activation of the Ndufs2-deficient and O\(_2\)-insensitive glomus cells by lactate (NADH accumulation and catecholamine release) remained unaltered (Fig. 6b–e). Moreover, CB cells from mice deficient of Hif2\(\alpha\), which have a normal MCI but strong inhibition of responsiveness to acute hypoxia due to down-regulation of atypical MCIV mitochondrial subunits\(^34\), also showed robust responses to lactate (Fig. 6f). These data indicate that lactate and oxygen sensing are mediated by separate mechanisms, although they share compartmentalized signals (rise of cytosolic NADH/NAD\(^+\) ratio and ROS) that induce depolarization, Ca\(^{2+}\) influx, and glomus cell transmitter release (Fig. 6g).

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In sum, we show here that the carotid arterial chemoreceptors play a major role in lactate homeostasis, a physiological process of broad functional and medical relevance. CB chemoreceptor cells
are activated by systemic hypoxia and thereby reduce the intensity of hypoxia-induced lactatemia. In addition, glomus cells are lactate sensors because they efficiently take up and metabolize lactate to increase the cytosolic NADH/NAD\(^+\) ratio and mitochondrial ROS production, which modulate the activity of membrane ion channels. Both lactate and hypoxia, although sensed by separate mechanisms, converge on producing cell depolarization and opening of Ca\(^{2+}\) channels (see Fig. 6g) and, therefore, have additive effects on glomus cells. The potentiation of CB activation by lactate during hypoxia facilitates a rapid reflex ventilatory compensation of hypoxemia and, secondarily, blunts peripheral lactate release (Fig. 7h). This adaptive response could minimize potential deleterious effects due to lactate release (e.g., metabolic acidosis) or excessive lactate uptake by cells. CB
activation by lactate may also contribute to hyperventilation and lactate homeostasis during physical exercise. The potential side-effects of CB resection or denervation on lactate homeostasis should be considered, as this surgical procedure is used in tumor surgery and is under experimental scrutiny for the treatment of sympathetically mediated diseases.

Methods

Experimental mice and rat models. Most of the experiments were performed in wild type adult mice, 2–3-month old without establishing differences in sex. Some experiments were performed in TH-NDUFS2, TH-HIF2a, and ERT2-HIF2a mice, which have previously been used in our laboratory. In addition, we used the TRPC5 (12031/Sv/ImJ) and TRPC6 (B6;129S8-Tpc6tm1Lbi/Mmjx) knockout mice purchased from The Jackson Laboratory. Double TRPC5/6 knockout mice were generated in our laboratory. Genotyping of TRPC5, TRPC6, and TRPC5/6 mice was done by PCR using different primers to detect each allele.

Preparation of adrenal medulla slices, dispersed chromaf fraction, and oIMR7415. 5-month-old male Wistar rats were sacrificed by intraperitoneal administration of a lethal dose of sodium thiopental (200 mg/kg) to dissect tissues for in vitro experiments. A few experiments were performed using 150 mg/kg of thiopental sodium as a sedative.

Preparation of cortical slices. Cortex (200–250 μm thick) was cut with a vibratome (VT1000S, Leica) in cold Tyrode’s solution, containing 120 mM NaCl, 5.4 KCl, 26 NaHCO3, 1.25 CaCl2, 1.25 MgCl2, 5 glucose, and 5 sucrose, at 37 °C. The slices were maintained on ice-cooled Tyrode’s solution and then placed on 35-mm culture dishes and maintained at 37 °C and 5% CO2 in growth medium (L-15 medium, Sigma, St. Louis, MO) and 2 mL L-glutamine (BioWhittaker, Velviers, Belgium), 10% fetal bovine serum (BioWhittaker, Velviers, Belgium), and 1% penicillin-streptomycin (BioWhittaker).

Preparation of glomus cells. Cells from glomus nodules were obtained from 3-month-old rats or mice by mechanical dispersion using the following protocol. Glomus nodules were minced with razor blades and incubated for 15 min in collagenase- and trypsin-containing solution to release peripheral capillary blood vessels (150 U/ml of collagenase type I A (Sigma, St. Louis, MO) and 150 U/ml of trypsin (Sigma)). After 15 min of incubation, the cell suspension was centrifuged at 37 °C for 5 min, and the pellet was resuspended in 100–150 μl of culture medium and plated on glass coverslips treated with poly-l-lysine (1 mg/ml) (Sigma). Cells were then incubated at 37 °C on a 5% CO2 incubator. Isolated cells were used for experiments up to 4–5 h after dispersion.

Preparation of cortical slices. Cortex (200–250 μm thick) was cut with a vibratome (VT1000S, Leica) in cold Tyrode’s solution, containing 120 mM NaCl, 5.4 KCl, 26 NaHCO3, 1.25 CaCl2, 1.25 MgCl2, 5 glucose, and 5 sucrose, at 37 °C. The slices were maintained on ice-cooled Tyrode’s solution and then placed on 35-mm culture dishes and maintained at 37 °C and 5% CO2 in growth medium (L-15 medium, Sigma, St. Louis, MO) and 2 mL L-glutamine (BioWhittaker, Velviers, Belgium), 10% fetal bovine serum (BioWhittaker), and 1% penicillin-streptomycin solution. The cells were then incubated at 37 °C on a 5% CO2 incubator. Isolated cells were used for experiments up to 4–5 h after dispersion.

Phytoplasmography. To study respiratory function, awake unrestrained mice were placed inside phytoplasmography chambers (EMKA Technologies) following a procedure adapted to our laboratory. Chambers were perfused with normal air (21% O2, normoxia), 10% O2 (hypoxia), or 5% CO2 (hypercapnia). The phytoplasmic stimulus was maintained during 5 minutes once O2 percentage reached 10% and the hypercapnic stimulus was maintained during 1 min when CO2 percentage reached 5% CO2. Both O2 and CO2 tensions were continuously monitored and recorded during the experiments.

Measurement of blood lactate. Lactate plasma concentration was determined using the Lactate Plus Meter, a hand-held testing device (Nova Biomedical). Two volumes of solution (1:1, 1.6 and 4:5, 6:6 ml lactate) were used to calibrate the device. To determine blood lactate concentration, a small drop of blood obtained from the mouse tail after a lancet puncture, was placed on the slot of specific strips for lactate quantification. Lactate measurement was performed without normoxia (21% O2), hypoxia (different oxygen concentrations were used: 15% O2 and 10% O2), and hypercapnia (5% CO2). All the measurements were done in a chamber designed for chronic hypoxia, where gas concentration was easily changed and monitored by specific O2 and CO2 sensors.

Recording solutions. For in vitro recordings, either CB, AM, or SCG dispersed cells or CB or AM slices, were transferred to the recording chamber and continuously perfused with a control extracellular solution containing, in mM: 117 NaCl, 4.5 KCl, 23 NaHCO3, 1 MgCl2, 2.5 CaCl2, 5 glucose, and 5 sucrose, at −35 °C. In solutions with 40 mM K+ or different lactate/pyruvate concentrations NaCl was replaced equimorally with KCl, sodium l-lactate or sodium pyruvate respectively. α-ketobutyrate (aKB) was added to the external solution at the indicated concentration.
concentrations. When N-methyl-D-glucamine (NMDG) was used extracellular Na⁺ was also equimolarly reduced. The “normoxic” solution was bubbled with a gas mixture of 2% CO₂, 5% O₂, and 93% N₂, whereas the “hypoxic” solution was bubbled with 5% CO₂ and 95% N₂ to reach an O₂ tension of <15 mm Hg in the recording chamber. The “hypercapnic” solution was bubbled with 20% CO₂, 20% O₂, and 60% N₂. Osmolality of solutions was <300 mOsm/kg and the pH was 7.4. All the pharmacological drugs used (nifedipine, AR-C155858; 2′-APB, Pr3Y), FTC720, and amiloride were dissolved in stock solutions before they were added directly to the external solution. Concentrations used are indicated in the figures and/or figure legends.

Amperometric recording of single cell catecholamine secretion in slices. Catecholamine secretion from glomus cells or chromaffin cells in CB or AM slices, respectively, was performed following a procedure developed in our laboratory. 64,66 Secreton fusions were detected with a 10 μm carbon fiber electrode. Amperometric currents were recorded with an EPC-8 patch clamp amplifier (HEKA Electronics), filtered at 100 Hz and digitized at 250 Hz before storage on computer. Data acquisition and analysis were performed with an ITC-16 interface (Instrutech Corporation) and PULSE/PULSEFIT software (HEKA Electronics). The amount of charge transferred to the recording electrode during a given period of time was calculated from the amount of charge transferred to the recording electrode during a given period of time. The subtraction of the rate of background current (mitochondrial ROS production) was performed following methods adapted to our experiments. The secretion rate (femtocoulombs (fC)/min) was calculated as the amount of charge transferred to the recording electrode during a given period of time.

**Ca²⁺, NADH, pH, and ROS measurement by single cell microfluorimetry.** Microfluorimetric measurements in single dispersed glomus cells, chromaffin cells or SCG neurons (intracellular Ca²⁺; pH, and NADH) or cells in CB slices (mitochondrial ROS production) was performed following methods adapted to our laboratory. 67–69,70 For these dispersed glomus cells suspended in an inverted microscope (Nikon eclipse Ti) equipped with a 40x/0.60 NA objective, a monochromator (Polychrome V, Till Photonics), and a CCD camera, controlled by Aquacosmos software (Hamamatsu Photonics). To study glomus cells in slices the system used consists of a directed microscope (Olympus, U-TV1x-2) equipped with a 60x/0.90 NA water immersion objective, a monochromator (Polychrome V, Till Photonics), and a CCD camera, controlled by Live Acquisition software (TILL Photonics). For the experiments, dispersed cells or carotid body slices were transferred to the recording chamber and perfused with the solutions described above. All experiments were performed at 35 ± 0.5 °C.

Cytoplasmic Ca²⁺ signals were digitized at a sampling interval of ≥500 ms. NADH microfluorimetric measurements (NADH/P1 autofluorescence) were performed in CB, AM or SCG dispersed cells using a non-ratiometric protocol widely used to estimate changes in NADH/P1 levels in CB cells. 44,47,48 NADH was excited at 360 nm and measured at 460 nm. The acquisition protocol was designed with a spatial resolution of 4 x 4 pixels, an excitation time of 150 ms, and then transferred to the recording chamber with a continuous solution (sheep, 1:200 dilution, AB1542, Millipore/Merck), and GFAP (chicken, 1:500, 69656, Novus Biologicals), MCT2 (rabbit, 1:500 dilution, PA5-77498, ThermoFisher Scientific), MCT4 (rabbit, 1:100 dilution, 22787-1-AP, ProteinTech), TH (sheep, 1:200 dilution, AB1542, Millipore/Merc), and GEFAP (chicken, 1:500, ab4674, Abcam). The secondary antibodies were: Alexa Fluor 568 (anti-rabbit, 1:400 dilution, A-11011, ThermoFisher Scientific), Alexa Fluor 488 (anti-sheep, 1:400 dilution, A-11015, Thermofisher Scientific), and Alexa Fluor 488 (anti-chicken, 1:400 dilution 103-545-155 Jackson ImmunoResearch). In addition, nuclei were labeled with 4′,6″-diamidino-2-phenylindole (DAPI, 1:1000 dilution).

Immunohistochemical analysis. For Immunofluorescent studies, mice were perfused first with PBS and then with 4% paraformaldehyde in PBS before tissue dissection. Carotid bifurcations and adrenal glands were fixed with 4% paraformaldehyde in PBS for 2 h, cryoprotected overnight with 30% sucrose in PBS, and embedded in OCT (Tissue-Tek). Tissue sections of 8 μm were obtained with a cryostat (Leica). These sections were incubated first with primary antibodies overnight at 4 °C, then with fluorescent secondary antibodies at room temperature for 1 h. The primary antibodies used were: MCT1 (rabbit, 1:200 dilution, NP-153, Novus Biologicals), MCT2 (rabbit, 1:500 dilution, PA-77498, ThermoFisher Scientific), MCT4 (rabbit, 1:100 dilution, 22787-1-AP, ProteinTech), TH (sheep, 1:200 dilution, AB1542, Millipore/Merc), and GEFAP (chicken, 1:500, ab4674, Abcam). The secondary antibodies were: Alexa Fluor 568 (anti-rabbit, 1:400 dilution, A-11011, Thermofisher Scientific), Alexa Fluor 488 (anti-sheep, 1:400 dilution, A-11015, Thermofisher Scientific), and Alexa Fluor 488 (anti-chicken, 1:400 dilution 103-545-155 Jackson ImmunoResearch). In addition, nuclei were labeled with 4′,6″-diamidino-2-phenylindole (DAPI, 1:1000 dilution).

Immunofluorescent images were obtained using Nikon A1R + confocal microscope. When necessary, orthogonal projection from Z-stack images was also presented in order to study potential colocalization between two proteins in the same cell.

Statistical analysis. Normality of the data sets obtained in the experiments was tested with the Shapiro–Wilk test. When necessary, a log transformation was performed to normalize the distribution prior to parametric analyses. Except in a few cases, all data in the figure legends, data had a normal distribution and were described as mean ± SEM with the number (n) of experiments indicated. Data from two groups were analyzed with either a two tails t test or a paired two tails t test. Data with multiple groups were analyzed by ANOVA or RM-ANOVA followed by a post-hoc Tukey’s test. For graphical representation of the data we used bar diagrams, with indication of the mean ± SEM, and a scatter plot of the data points superimposed. For the sake of clarity, we used for graphical representation box plots, with indication of median, quartiles, and outliers, in panels where all data sets had n ≥ 10 data points. In these last cases mean ± SEM values are given in the figure legends. For data without a normal distribution the analysis of statistically significant differences between different conditions was done with non-parametric tests (Mann–Whitney rank-sum test or Kruskal–Wallis test, followed by a post-hoc Tukey’s test). For graphical representation of non-parametric data, we used box plots with indication of median, quartiles, and outliers. Mean ± SEM values in these data sets are also given in the figure legends. Statistical analyses were done using Prism Version 8.2.1. (279) for MacOS. A P < 0.05 or smaller was considered statistically significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Data generated or analyzed over the course of this study are included within the paper. The data that support the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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**Patch clamp recordings in dispersed carotid body glomus cells and SCG neurons.** Membrane potential was recorded from dispersed mouse glomus cells and SCG neurons using a whole-cell configuration using the standard voltage-clamp technique as adapted in our laboratory. 40,70. Patch clamp pipettes (2–4 MΩ) were pulled from capillary glass tubes with a horizontal pipette puller P-1000 (Sutter instruments) and fire-polished with a microforge MF-830 (Narishige). Current-clamp recordings were obtained with an EPC-7 amplifier (HEKA Electronik). The signal was filtered (3–10 kHz), subsequently digitized with an analog/digital converter ITC-16 (Instrutech Corporation) and finally sent to the computer. Data acquisition and storage were performed using the Pulse/Pulsefit software (HEKA Electronics) at a sampling interval of 4 ms. Data analysis were performed using the Igor Pro Carbon (WaveMetrics) and Pulse/Pulsefit (HEKA Electronik) programs. For perforated patch experiments the pipette solution contained (in mM): 70 KSO₄, 30 KC1, 2 MgCl₂, 1 EGTA, 10 HEPES, pH 7.2. Amphotericin B (240 μg/ml) was added to this solution. Bath solutions composition was described in “Recording solutions.”
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**Author contributions**

H.T.-T., P.O.-S., and L.G. performed the experiments and analyzed the data. P.O.-S., H. T.-T., and J.L.-B. designed the study and contributed to generating the draft of the manuscript. J.L.-B. coordinated the project and the writing of the paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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