

UNIVERSITY OF BIRMINGHAM

University of Birmingham
Research at Birmingham

Adrenaline activation of the carotid body: key to CO₂ and pH homeostasis in hypoglycaemia and potential pathological implications in cardiovascular disease

Holmes, Andrew; Ray, Clare; Thompson, Emma; Alshehri, Ziyad; Coney, Andrew; Kumar, Prem

DOI:

[10.1016/j.resp.2018.05.008](https://doi.org/10.1016/j.resp.2018.05.008)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Holmes, A, Ray, C, Thompson, E, Alshehri, Z, Coney, A & Kumar, P 2018, 'Adrenaline activation of the carotid body: key to CO₂ and pH homeostasis in hypoglycaemia and potential pathological implications in cardiovascular disease', *Respiratory physiology & neurobiology*. <https://doi.org/10.1016/j.resp.2018.05.008>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

<https://doi.org/10.1016/j.resp.2018.05.008>

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

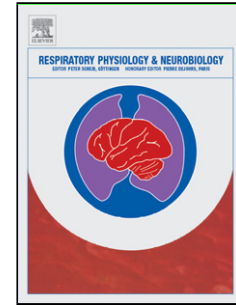
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Accepted Manuscript

Title: Adrenaline activation of the carotid body: key to CO₂ and pH homeostasis in hypoglycaemia and potential pathological implications in cardiovascular disease

Authors: Andrew P. Holmes, Clare J. Ray, Emma L. Thompson, Ziyad Alshehri, Andrew M. Coney, Prem Kumar



PII: S1569-9048(18)30052-1
DOI: <https://doi.org/10.1016/j.resp.2018.05.008>
Reference: RESPNB 2970

To appear in: *Respiratory Physiology & Neurobiology*

Received date: 13-2-2018
Revised date: 17-5-2018
Accepted date: 21-5-2018

Please cite this article as: Holmes, Andrew P., Ray, Clare J., Thompson, Emma L., Alshehri, Ziyad, Coney, Andrew M., Kumar, Prem, Adrenaline activation of the carotid body: key to CO₂ and pH homeostasis in hypoglycaemia and potential pathological implications in cardiovascular disease. *Respiratory Physiology and Neurobiology* <https://doi.org/10.1016/j.resp.2018.05.008>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Adrenaline activation of the carotid body: key to CO₂ and pH homeostasis in hypoglycaemia and potential pathological implications in cardiovascular disease.

Andrew P. Holmes ¹, Clare J. Ray ¹, Emma L. Thompson ¹, Ziyad Alshehri ¹, Andrew M. Coney ¹, Prem Kumar ¹

¹ Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK, B12 2TT

Word count: 5318

Corresponding author: P Kumar

Email: p.kumar@bham.ac.uk

Table of contents category: Respiratory

Highlights

- Carotid body activation during hypoglycaemia is essential for hyperpnoea thereby protecting against a rise in CO₂ and systemic acidosis.
- Our recent findings reveal that the counter-regulatory hormone adrenaline is a novel and important physiological stimulus for the carotid body in hypoglycaemia.
- Identification of adrenaline as a physiological activator of CB function raises the intriguing possibility that carotid body activation and hyperpnoea may be necessary to maintain pH in other in other adrenaline-related hypermetabolic states such as exercise.

- Furthermore, repetitive exposure to hypoglycaemia and adrenaline or a chronic rise in adrenaline as occurs in type 1 diabetes and sleep disordered breathing/heart failure respectively, may have the potential to cause chronic pathological changes in carotid body function contributing to carotid body-mediated neurogenic hypertension.
- Understanding better a potential pathological role of adrenaline could open new avenues for treating carotid body mediated cardiovascular disease.

Abstract

Ventilatory and neuroendocrine counter-regulatory responses during hypoglycaemia are essential in order to maintain glycolysis and prevent rises in $P_a\text{CO}_2$ leading to systemic acidosis. The mammalian carotid body has emerged as an important driver of hyperpnoea and glucoregulation in hypoglycaemia. However, the adequate stimulus for CB stimulation in hypoglycaemia has remained controversial for over a decade. The recent finding that adrenaline is a physiological activator of CB in hypoglycaemia raises the intriguing possibility that CB stimulation and hyperpnoea may be necessary to maintain pH in other adrenaline-related hypermetabolic states such as exercise. This review will therefore focus on 1) The important functional contribution of the CB in the counter-regulatory and ventilatory response to hypoglycaemia, 2) the proposed mechanisms that cause CB stimulation in hypoglycaemia including hormonal activation by adrenaline and direct low glucose sensing and 3) the possible pathological consequences of repetitive CB activation by adrenaline that could potentially be targeted to reduce CB-mediated cardiovascular disease.

Key words: Adrenaline, carotid body, hypoglycaemia, glucose, CO_2 , pH, hypercapnia

1. Introduction

Precise control of arterial blood glucose is a fundamental regulatory process that allows for continuous maintenance of glycolysis in all cell types. Dysfunctional glucoregulation in patients with either type 1 or type 2 diabetes increases the risk of hypertension, coronary artery disease, heart failure, cardiac arrhythmia, renal disease, neuropathy, retinopathy, hospitalisation and death (Atkinson et al., 2014; Bartnik et al., 2004; Bell, 2003; Maahs et al., 2005; Soedamah-Muthu et al., 2006). Furthermore, severe iatrogenic insulin-induced hypoglycaemia in type 1 diabetics has the potential to cause coma and even death (Boland et al., 2001; Cryer et al., 2003). The counter-regulatory responses to hypoglycaemia is dependent on the stimulation of multiple peripheral and central glucose sensors located in the gastrointestinal tract, portal mesenteric vein, hypothalamus and hindbrain (Donovan and Watts, 2014). Integration of this information drives a number of important neuro-endocrine responses including adrenaline (Adr) release from the adrenal medulla (ADM) and inhibition of insulin and stimulation of glucagon secretion from pancreatic islet β - and α -cells respectively (Balfour et al., 2006). Recent evidence suggests that the carotid body (CB) chemoreceptors, situated at the carotid bifurcation, also play an important role in the counter-regulatory response to hypoglycaemia, both in terms of re-establishing normal blood glucose levels and in augmenting ventilation to match the increase in metabolic rate to preserve P_aCO_2 , pH and prevent acidosis. This review will focus on 1) The important functional contribution of the CB in initiating some of the reflex responses to hypoglycaemia, 2) the proposed mechanisms that cause CB stimulation in hypoglycaemia including hormonal activation by Adr and insulin or direct low glucose sensing and 3) the possible pathological consequences of repetitive CB activation by hormones that could promote development of novel treatments for CB-mediated cardiovascular disease.

2. Carotid body regulation of arterial blood glucose

There is now an emerging body of evidence highlighting the important functional role for the CB chemoreceptors in restoring blood glucose levels in response to acute hypoglycaemia. The idea that the CB could be involved in glucose homeostasis was first triggered by the observations that direct excitation of the CB using cyanide (a metabolic poison and well-known stimulus for the CB) causes reflex hyperglycaemia in anaesthetised cats (Alvarez-Buylla and de Alvarez-Buylla, 1988). This CB-mediated reflex pathway is reliant on chemoafferent input into the nucleus tractus solitarius (NTS) and an increase in sympathetic activity into the adrenal medulla, thereby increasing Adr secretion and hepatic glucose release from stored glycogen (Alvarez-Buylla et al., 1997; Alvarez-Buylla and de Alvarez-Buylla, 1988). Integration of the chemoafferent signal in the NTS involves both stimulated neuronal release of NO and inhibition of GABAergic interneurons (Lemus et al., 2008; Montero et al., 2014). These findings have since been extended in important studies by Koyama and colleagues, who demonstrated that in conscious dogs, CB resection significantly elevates the required rate of glucose infusion to sustain a fixed blood glucose concentration during insulin-induced hypoglycaemic clamp (Koyama et al., 2000), thus revealing that CB resection removes a vital functional component in the counter-regulatory response to hypoglycaemia. These CB-resected animals also exhibit a reduced level of endogenous hepatic glucose release, consistent with a central role for the CB in promoting neuro-endocrine responses during hypoglycaemia (Koyama et al., 2000). In humans, silencing of CB by hyperoxia reduces endogenous glucose release during hypoglycaemia and decreases the secretion rate of counter-regulatory hormones including Adr, noradrenaline (NA), cortisol, growth hormone and glucagon (Wehrwein et al., 2010). Furthermore, in chronic CB-resected patients, the reduction in hypoxic ventilatory response (HVR) directly correlates with a decrease in counter-regulatory response to hypoglycaemia (Wehrwein et al., 2015). These findings underline an important function of the CB in promoting counter-regulatory hormone secretion and glucose release during hypoglycaemia in humans.

3. Carotid body preservation of PCO₂ and pH during acute hypoglycaemia

Preservation of pH during acute hypoglycaemia is an important and often overlooked regulatory process. Hypoglycaemia and counter-regulatory secretion of Adr and NA, act to drive up whole body metabolic rate and the CO₂ generation (Bin-Jaliah et al., 2004). Thus, hyperpnoea is necessary to avoid excessive build-up of arterial PCO₂ and a systemic acidosis. Indeed, the complete absence of ventilatory response to hypoglycaemia can be fatal (Bin-Jaliah et al., 2004). It is now clear that hyperpnoea in hypoglycaemia is dependent on stimulation of the CB, as evidenced by CB resection preventing an increase in minute ventilation (V_E) during insulin-induced hypoglycaemic clamp protocols in anaesthetised rats (Bin-Jaliah et al., 2004, 2005; Thompson et al., 2016). Furthermore, CB stimulation evokes a precise matching of the increase in V_E with O₂ consumption such that the $V_E:VO_2$ ratio remains constant (Bin-Jaliah et al., 2004). Interestingly, moderate systemic hypoglycaemia e.g. as occurs in short term fasting, at a level that does not elevate whole body metabolic rate, does not augment ventilation in conscious mice (Ohshima et al., 2011). This suggests that it is the rise in metabolic rate and not hypoglycaemia *per se* that is required to stimulate breathing (Kumar, 2007). This has since been supported by the finding that blockade of adrenergic signalling during hypoglycaemia, either by propranolol or adrenalectomy, produces hypoventilation and a rise in arterial PCO₂ in anaesthetised rats (Thompson et al., 2016).

Activation of the CB during hypoglycaemia is associated with an increase in CO₂ sensitivity (Bin-Jaliah et al., 2005; Thompson et al., 2016). Hypoglycaemia increases the whole body ventilatory responses to hypercapnia in anaesthetised rats, an effect that is abolished by CB resection (Bin-Jaliah et al., 2005). Again, this seems to be dependent on the release of Adr from the adrenal medulla and activation of β -adrenoreceptors (Thompson et al., 2016). Moreover,

exposure of isolated CB type I cells to physiological concentrations of Adr increase $[Ca^{2+}]_i$ release responses to hypercapnia, signifying heightened CO_2 sensitivity. A feed-forward mechanism of this type involving augmented CB CO_2 sensitivity during Adr-mediated hypermetabolism allows for ventilation to increase in proportion with the rise in metabolism, without any initial elevation or fluctuation in P_aCO_2 , thus allowing for more precise control of blood pH. Whether or not this same mechanism is physiologically relevant in other Adr-mediated hypermetabolic states, such as exercise, where hyperpnoea is necessary to maintain P_aCO_2 and pH, warrants future consideration. It is interesting to point out that further to their initial study Koyama et al. did demonstrate that during exercise, plasma glucose concentration fell below control levels in CB-resected conscious dogs immediately after onset of exercise, and remained significantly lower throughout (Koyama et al., 2001). In a more recent study, targeting the CB using dopamine significantly reduced blood glucose levels in humans during exercise (Johnson et al., 2018). These findings suggest that CB activation in exercise is important in preserving blood glucose concentration. Exploring the mechanism of CB activation and its role in hyperpnoea in exercise remains to be elucidated.

In addition to heightened CO_2 sensitivity, there is also evidence to suggest that the *in vivo* hypoxic ventilatory sensitivity is augmented during hypoglycaemia in humans (Ward et al., 2007). Given the importance of the CB in evoking the HVR, it is tempting to speculate that this is due to an elevation in CB O_2 sensitivity. This is still to be verified *in vivo* or *in vitro*. In the study by Ward et al. (2007), the augmentation in HVR was associated with a concurrent rise in glucagon, Adr, NA and cortisol. Interestingly, after removal of the hypoglycaemic clamp the HVR remained elevated. Analysis of serum hormones revealed that NA and cortisol had not returned to baseline, possibly indicative of a putative link between these hormones and elevated

CB O₂ sensitivity (Ward et al., 2007). Again, the presence of such a mechanism still needs to be confirmed in both humans and animals.

4. Carotid body stimulation in hypoglycaemia: hormonal activation or direct sensing of low glucose?

Although there is little doubt that the CB is stimulated in hypoglycaemia, the adequate stimulus has remained highly controversial for over a decade. Currently there are 3 proposed stimuli for CB excitation in insulin-induced hypoglycaemia, those being 1) low glucose, 2) insulin and 3) adrenaline.

4.1 Direct sensing of low glucose by the CB

The first study proposing inherent low glucose sensing of the CB was performed using CB slices isolated from rats (Pardal and Lopez-Barneo, 2002). In these studies, the authors reported that glucose deprivation evoked significant dopamine (DA) release from cultured CB slices. This response was dependent on inhibition of outward iberiotoxin sensitive K⁺ current over a range of -20 to +40mV. Glucose deprivation also augmented the CB slice response to hypoxia (Pardal and Lopez-Barneo, 2002). Similar findings have now been reported in human CB slices (Ortega-Saenz et al., 2013). Furthermore, experiments focusing on the mechanisms of low glucose sensing have since revealed that glucose deprivation produces type I cell depolarisation, increases [Ca²⁺]_i and activates a background Na⁺ current (Garcia-Fernandez et al., 2007). These initial observations have been strengthened by the finding that another CB preparation; co-cultures of rat type I cells and chemoafferent petrosal neurones, respond rapidly to low glucose (Zhang et al., 2007). In this preparation, low glucose causes a concentration dependent rise in chemoafferent spike frequency over a physiological glucose range. The authors also identified

that neurotransmission was through co-release of ATP and ACh and verified the proposal that low glucose heightens the CB response to hypoxia.

In contrast to other recognised glucose sensors, the CB slice response to low glucose seems to be independent of any change in ATP, since responses to glucose deprivation are still apparent even when intra-cellular ATP is clamped (Garcia-Fernandez et al., 2007). This also suggests that responses to low glucose are dissimilar to hypoxia, the classical stimulus of the CB that commonly is reported to be dependent on a reduction in mitochondrial function and changes in intracellular ATP or MgATP concentration (Buckler and Turner, 2013; Duchen and Biscoe, 1992a, b; Holmes et al., 2016; Varas and Buckler, 2006). Furthermore, rat CB type I cells lack expression of other important markers of specialised glucose sensitive cells including GLUT-2 and glucokinase (Dunn-Meynell et al., 2002; Garcia-Fernandez et al., 2007; Thorens and Mueckler, 2010). The rodent CB does express the AMP-activated protein kinase (AMPK) (Mahmoud et al., 2016; Wyatt et al., 2007), an enzyme reported to be involved in glucose chemotransduction signalling in glucose sensitive neurones in the arcuate nucleus (Han et al., 2005; Kim et al., 2004; Murphy et al., 2009). However, AMPK stimulation by low glucose requires a fall in $[ATP]_i$, or at least a shift to an increase in the $[AMP]/[ATP]$ ratio caused by metabolic stress (Han et al., 2005; Kim et al., 2004; Murphy et al., 2009). With metabolic stress and/or a fall in ATP previously ruled out by earlier studies performed by Garcia-Fernandez and colleagues, it is unlikely that AMPK is involved in the CB slice response to glucose deprivation. Therefore, current evidence suggests that the CB is different to other important central and peripheral gluco-sensors but as yet an intracellular low glucose-sensing molecule (or a glucose receptor) in the CB remains elusive (Figure 1).

Despite this elegant and provocative work, the idea that the CB functions as a physiological low glucose sensor is not universally accepted. This is due to a number of different *in vitro* CB preparations exhibiting a complete lack of intrinsic low glucose sensitivity. The acutely isolated intact rat CB is completely unresponsive to fluctuations in the superfusate glucose concentration. This is based on findings that 2mM glucose or even complete glucose deprivation fails to increase chemoafferent frequency in this intact rat CB preparation (Bin-Jaliah et al., 2004; Holmes et al., 2012; Holmes et al., 2014). Accordingly, it has been shown that severe low glucose (1mM) or glucose deprivation do not significantly induce catecholamine, ATP or ACh release, in similar intact rat and cat CB preparations (Conde et al., 2007; Fitzgerald et al., 2009). Furthermore, low glucose or glucose deprivation does not augment CB chemoafferent or neurosecretory responses to acute hypoxia or hypercapnia (Conde et al., 2007; Holmes et al., 2012; Holmes et al., 2014).

A similar absence of intrinsic low glucose sensitivity has been reported in freshly dissociated rat CB type I cells. Background TASK-like channel current, an important effector of O₂, CO₂ and acid sensing in the CB, is completely unaffected by glucose deprivation (Kim et al., 2011). Furthermore, the ATP sensitive K⁺ (K_{ATP}-like) current (distinguishable from TASK) is also reported to be completely insensitive to the removal of superfusate glucose (Kim et al., 2011). This may be of particular significance given the importance of K_{ATP} channels in well-established glucose sensitive cells in the brain, pancreas and portal vein. In addition, dissociated rat CB type I cells, used within hours of isolation, display no elevation in [Ca²⁺]_i in response to glucose free media (Gallego-Martin et al., 2012; Holmes et al., 2014). Consistent with the intact preparation, the type I cell response to hypoxia is not modified by glucose deprivation (Holmes et al., 2014).

Interestingly, an increase in DA secretion has been observed in the acutely isolated intact preparation, but only following 40 minutes of glucose deprivation (Conde et al., 2007). Moreover, chemoafferent discharge frequency does eventually increase in intact preparation, but again, only after at least 30 minutes of complete removal of glucose and other substrates from the superfusate (Holmes et al., 2014). This rise in sensory activity can be rapidly reversed by adding back just 1mM glucose or other substrates such as lactate and pyruvate. The time taken to respond to glucose deprivation can also be significantly reduced by pharmacological inhibition of type I cell glycogen metabolism (Holmes et al., 2014). Glycolysis is dependent on the enzymatic activity of hexokinases. The absence of glucokinase (hexokinase IV; a marker for glucose sensitive cells and highly sensitive to glucose with K_m approximately 8mM) from CB type I cells (Garcia-Fernandez et al., 2007), indicates that glycolysis will only run-down in the type I cells at very low concentrations of intracellular glucose, since all other hexokinases have a K_m for glucose in the μ M range (Lowry and Passonneau, 1964; Meglasson and Matschinsky, 1986). Reversal of chemoexcitation by adding back just 1mM glucose is therefore indicative of the actual response being a complete run-down in glycolysis and this takes place only after at least 30 minutes due to the presence of a functional glycogen store that can be mobilised under conditions of complete glucose deprivation. A mechanism of this type for sensing physiological glucose *in vivo* would be physiologically ineffective.

It has been shown that glycogen is located in type I cells in the CB (Holmes et al., 2014), but other sources could include the type II cell and nerve ending (Nishi and Stensaas, 1974; Vazquez-Nin et al., 1977). Interestingly, in both central and peripheral neuronal tissue it is the glial cells that store glycogen, which is metabolised to release lactate in order to fuel neuronal activity during periods of increased activity or hypoglycaemia (Brown et al., 2012; Brown and Ransom, 2007; Brown et al., 2005; Brown et al., 2003). As yet a functional role for type II cells

in preserving or mediating the CB response to hypoglycaemia is unknown but could be a good avenue for future investigation.

So, what is the reason for conflicting observations seen between different CB preparations? Is it possible that the more rapid responses to low glucose seen in either the CB slice or CB co-culture of type I cells and chemoafferent neurones is due to a change in metabolic status? These preparations are cultured over a period of days, often in high glucose media and hyperoxia (Pardal and Lopez-Barneo, 2002; Zhang et al., 2007), and the consequences of this on metabolic reserve or ability to metabolise glucose or glycogen are unknown. However, we speculate that this period and method of culture may make the cells/tissue more reliant on glycolysis and impair the glycogen store, thus making preparations more sensitive to a subsequent exposure to low glucose or glucose deprivation. Consistent with this idea, it has been demonstrated that rat CB tissue that initially shows an inherent lack of low glucose sensitivity immediately following isolation, does start to exhibit some degree of low-glucose sensitivity after a period of 24 hours in culture (Gallego-Martin et al., 2012). This again supports the idea that a change in metabolic status during long term culture starts to make the CB tissue more sensitive to changes in glucose.

4.2 Insulin stimulation of the CB

If the CB is unable to directly sense low-glucose, the question remains as to the stimulus that excites the CB during insulin-induced hypoglycaemia *in vivo*. Recent data suggests that insulin itself could act as the adequate stimulus (Ribeiro et al., 2013). The rat CB type I cells express insulin receptors and it has been reported that exogenous insulin increases type I cell $[Ca^{2+}]_i$ and amplifies ATP and DA release *in vitro* (Ribeiro et al., 2013) (Figure 1). Insulin also augmented type I cell responses to hypoxia. Furthermore, under conditions of euglycaemic clamp, insulin

elevates ventilation, an effect abolished by CSN section (Ribeiro et al., 2013). Recently, it has been observed that insulin elevates ventilation in humans independently of any alteration in blood glucose (Barbosa et al., 2018). A mechanism of this nature would explain CB activation in hypoglycaemia and possibly also an interaction between metabolic rate and CB stimulation. However, in contrast, a previous study found that a euglycaemic-hyperinsulinaemic clamp did not augment ventilation *in vivo* in anaesthetised rats (Bin-Jaliah et al., 2004), raising questions about a role for insulin in provoking CB chemoexcitation. Additionally, there is evidence that insulin can act centrally in the arcuate nucleus of the hypothalamus to stimulate sympathetic nerve activity (Cassaglia et al., 2011), independent of CB stimulation. Although intriguing, much is therefore still to be clarified about a role for insulin in modifying CB function, and we await characterisation of an insulin signal transduction cascade within the CB type I cell that heightens CO₂ or O₂ sensitivity (Figure 1).

4.3 Adrenaline activation of the CB

Alternatively, it has recently been proposed that Adr might be the adequate stimulus that augments CB activity in hypoglycaemia (Thompson et al., 2016). Numerous studies have demonstrated that exogenous Adr, NA or β -adrenoreceptor agonists augment ventilation in multiple species, an effect that is dependent on CB stimulation and CSN input into the central nervous system (Folgering et al., 1982; Hauton et al., 2013; Joels and White, 1968; Thompson et al., 2016). The threshold for an increase in ventilation by Adr seems to lie between 0.1 and 1 $\mu\text{g kg}^{-1} \text{ min}^{-1}$ infusion rate (Linton et al., 1992; Thompson et al., 2016), a concentration that we estimate would be in the low nM range, below that which causes an increase in blood pressure. Adr potentiates the ventilatory response to hypercapnia acting through β -adrenoreceptors, and this response does appear to be blunted by hyperoxia consistent with the recognised CO₂-O₂ interaction in the CB (Dasso et al., 2000; Joels and White, 1968; Pepper et

al., 1995; Thompson et al., 2016). When administered at a physiological level (1-10nM) Adr also directly heightens rat CB type I cell CO₂ sensitivity *in vitro* (Thompson et al., 2016). At much higher doses (greater than 1µM) Adr starts to inhibit CSN activity, possibly a consequence of non-selective dopamine D₂ receptor activation (Hauton et al., 2013).

Adr is released by the adrenal medulla during hypoglycaemia. Hyperpnoea in insulin-induced hypoglycaemia in anaesthetised rats can be completely blocked by propranolol and adrenalectomy, as well as by section of the CSN (Bin-Jaliah et al., 2004; Thompson et al., 2016). Whilst pointing towards a mechanism of Adr-induced activation of CB β-adrenoreceptors, these findings again challenge the notion of low glucose or insulin acting directly on the CB. Adr release from the adrenal medulla also enhances ventilatory CO₂ sensitivity, again conferred through β-adrenoreceptor stimulation (Thompson et al., 2016). Thus, a β-adrenoreceptor mediated stimulation of the CB appears to be necessary in resetting CO₂ sensitivity, driving hyperpnoea and ensuring pH preservation in hypoglycaemia. However, the presence and relative abundance of multiple different β-adrenoreceptor subtypes in the CB is still to be clarified. This mechanism of CB mediated hyperpnoea in hypoglycaemia mediated by Adr is summarised in Figure 1.

These studies are the first to identify physiological functional roles of Adr in terms of causing CB excitation and increasing ventilation. Yet, as with insulin, there is still much to uncover, especially the precise mechanisms accounting for type I cell excitation by Adr. Given the exceptionally high level of G-protein coupled receptor subunits recently identified by whole CB and type I cell RNA sequencing, and the presence of numerous transmembrane adenylyl cyclase receptor subtypes and phosphodiesterases, it is likely that Adr acts at least in some part by modifying type I cell cAMP (Chang et al., 2015; Nunes et al., 2010; Nunes et al., 2013; Zhou et

al., 2016). cAMP can be modulated by numerous other neurotransmitters and neuromodulators in the CB and can modify type I cell hypoxic and hypercapnic sensitivity by interactions with exchange protein activated by cAMP (EPAC) or protein kinase A (PKA) (Holmes et al., 2015; Holmes et al., 2017; Nunes et al., 2014; Rocher et al., 2009; Salman et al., 2017; Xu et al., 2006; Zhang et al., 2017). Yet a role for cAMP in CB stimulation during hypoglycaemia or over-stimulation in CB related pathology is still to be fully explored (Nunes et al., 2014). Furthermore, the possibility that Adr/cAMP signalling in the CB is important for matching ventilation with metabolism in other common circumstances such as exercise or psychological stress warrants further study.

A summary of the three proposed mechanisms by which hypoglycaemia leads to carotid body activation and hyperpnoea is summarised in Figure 1.

5. Carotid body dysfunction in metabolic and cardiovascular disease; potential roles for hormonal activation

5.1 Sleep disordered breathing

In the Western World, the prevalence of sleep disordered breathing (SDB) in the middle aged or elderly is 9-26% in males and 9-28% in females (Duran et al., 2001; Young et al., 1993; Young et al., 2002). SDB is expected to continue to rise in both the UK and Western populations due to the rapid elevation in childhood obesity over the last 25 years (Statistics supplied by WHO and Public Health England). SDB is characterised by periods of apnoea or hypopnoea ($\geq 30\%$ airflow cessation) occurring at a rate of ≥ 5 events per hour, with each being accompanied by a $\geq 4\%$ decrease in oxyhaemoglobin saturation (Flemons et al., 1999; Nieto et al., 2000). Patients with SDB have a higher incidence of hypertension, coronary artery disease (CAD), chronic

heart failure (CHF), atrial fibrillation (AF), stroke and death (Gami et al., 2007; Kanagala et al., 2003; Nieto et al., 2000; Peppard et al., 2000; Shahar et al., 2001; Stevenson et al., 2008; Yaggi et al., 2005; Yaranov et al., 2015). A key component of SDB in patients is the elevation in resting sympathetic nerve activity mediated by a dysfunctional CB (Carlson et al., 1993; Narkiewicz et al., 1998; Somers et al., 1995). Similar findings have been reported rodent models of SDB (preconditioned with chronic intermittent hypoxia-CIH), with a pathological rise in CB activity accounting for increased resting sympathetic nerve activity, hypertension and cardiac arrhythmia (Del Rio et al., 2016; Fletcher et al., 1992; Peng et al., 2014a).

The proposed mechanism(s) leading to the chronic hyperactivity in the CB remain to be fully established. Much of the published work has focused on tonic up-regulation of reactive oxygen species (ROS) generation, dependent on 5-HT₂ receptor stimulation and increased expression and activity of NADPH oxidase 2 (NOX-2) (Peng et al., 2009; Peng et al., 2006a). The balance of hypoxia inducible factor (HIF-1 α and HIF-2 α) expression and activity within the type I cell is also important in conferring CB hyper-excitability following CIH (Peng et al., 2006b; Yuan et al., 2011; Yuan et al., 2008). In addition, it has been suggested that systemic and localised inflammatory mediators such as IL-1 β and TNF α are upregulated in response to CIH and contribute to the potentiation of the peripheral chemoreflex (Del Rio et al., 2012; Lam et al., 2012).

However, in SDB, repetitive CB stimulation by exposure to nightly CIH also causes a chronic rise in plasma Adr in humans, as evidenced by increased rates of urinary Adr excretion (Elmasry et al., 2002; Kelly et al., 2010; Marrone et al., 1993). These findings have been verified in animals exposed to CIH (Peng et al., 2014b; Prabhakar et al., 2012). Furthermore, recent evidence shows that baseline elevations in serum Adr, hepatic glucose release and fasting

hyperglycaemia evoked by CIH are dependent on increased CB activity (Shin et al., 2014). The novel finding that Adr is an important physiological activator of the CB (Thompson et al., 2016) raises the possibility that chronic exposure to high plasma Adr could be functionally relevant in heightening baseline CB sensory activity in this pathology, which in turn evokes chronic sympathetic activation and further Adr release from the adrenal medulla, in a positive feedback loop. There is already evidence showing that chronic exposure to exogenous β -adrenoreceptor agonists (isoprenaline-delivered by osmotic mini-pump) in rats causes hypertension, augments basal ventilation and exaggerates responses to both hypercapnia and hypoxia (Hauton et al., 2013). These observations are consistent with CB hyperactivity. Furthermore, in animal models, resection of the adrenal medulla prevents CIH-induced hypertension (Bao et al., 1997; Peng et al., 2014b). The precise mechanisms for this are unknown but could be related to a role for chronic catecholamine release in eliciting pathological changes in CB function. In addition, a number of common variants have been identified within or adjacent to β -adrenoreceptor gene loci, that associate with increased risk of hypertension and cardiovascular disease (Iwamoto et al., 2011; Johnson and Terra, 2002). The functional link between these polymorphisms and hypertension needs to be clarified to better define the disease mechanism and to predict personalised responses to anti-hypertension drug therapy. Nevertheless, this is an important area for future research and the clinical potential of the use of beta-blockers could be an intriguing possibility for selective treatment of hypertension in patients with SDB. However, a number of issues would need to be addressed to consider this as a potential therapeutic strategy, such as the possibility of β -adrenoreceptor internalisation, as occurs in heart failure (Lohse et al., 1996) and the impact of non-specific effects of beta-blockers such as inhibition of β -adrenoreceptor mediated bronchodilation.

5.2 Type 1 diabetes

Type 1 diabetes (T1D) is an autoimmune disorder commonly acquired in childhood that accounts for approximately 7-12% of all diabetes mellitus cases. By 2015, the number of patients (age 0-14) with T1D globally was 542,000 with approximately 86,000 newly diagnosed incidents each year. In the UK alone about 19,800 children under the age of 15 have T1D (International Diabetes Federation, 2015). T1D patients have a high incidence of major cardiovascular-related illnesses including hypertension, CAD, stroke, chronic kidney disease, heart failure, peripheral neuropathy and retinopathy (Atkinson et al., 2014; Maahs et al., 2005; Soedamah-Muthu et al., 2006). Emerging evidence demonstrates that the prevalence of SDB in adult T1D patients is remarkably high, reported to be between 10 and 50% (Borel et al., 2010; Manin et al., 2015; Schober et al., 2011). Furthermore, a recent study demonstrates that in T1D patients SDB is an independent risk factor for developing cardiovascular disease including hypertension, suggestive of an important interaction between these two morbidities (Manin et al., 2015). In addition, patients with T1D show pathological changes in autonomic balance characterised by reduced heart rate variability (HRV) and impaired baroreflex sensitivity (BRS) (Lanza et al., 2007; Limberg et al., 2015; Limberg et al., 2014). The mechanisms accounting for these important observations are still to be elucidated but could be related to further pathological modification in CB activity.

Importantly, adult and child T1D patients have the highest recurrence of asymptomatic hypoglycaemia, spending approximately 10% of total time with a plasma glucose concentration between 2.8 and 3.3mM (Boland et al., 2001; Cryer et al., 2003). Furthermore, T1D patients have on average 2 symptomatic episodes of hypoglycaemia per week, thousands per lifetime and one severe episode per year (Cryer et al., 2003). The recent data identifying that Adr, and insulin are both capable of stimulating the CB in hypoglycaemia could be important for this patient population (Ribeiro et al., 2013; Thompson et al., 2016). There is a possibility that

chronic intermittent hypoglycaemia in T1D patients might be sufficient to evoke pathological CB re-modelling predisposing to increased cardiovascular disease. Furthermore, given the high association between T1D and SDB (when plasma catecholamines are already elevated), the chronic intermittent episodes of hypoglycaemia and Adr release may exaggerate pathological CB re-modelling in this patient cohort (Figure 2). Future studies evaluating this directly in either humans or animal models may be of particular clinical importance.

5.3 Type 2 diabetes

Type 2 diabetes (T2D) is associated with poor glycaemic control, insulin resistance (IR) and remains one of the leading causes of cardiovascular disease (Inzucchi et al., 2012; Stumvoll et al., 2005). Recent data has identified that CB activity is augmented in rat models of IR induced by high calorific diet (Ribeiro et al., 2013). Early stage CB denervation prevents the development of hypertension and IR in this model (Sacramento et al., 2017). It has been proposed that hyperinsulinaemia, common in early stages of T2D and obesity, drives excessive CB and sympathetic activation thus predisposing to hypertension and IR (Conde et al., 2014; Ribeiro et al., 2013). In healthy humans, a recent study observed that increased body fat percentage and waist circumference is associated with a greater chemoreflex mediated blood pressure response to hypoxia, an effect that was correlated to plasma insulin concentration (Paleczny et al., 2016). Although suggestive of insulin augmenting CB activity, these findings should be treated with some caution since neither ventilatory nor heart rate responses to hypoxia were elevated in these same individuals despite having significantly higher plasma insulin concentrations. Given that T2D is also considered to be a chronic low grade inflammatory disease accompanied by increased Il-1 signalling (Dandona et al., 2005; Wellen and Hotamisligil, 2005), the action of local or systemic inflammatory mediators could be an alternative mechanism that causes an increase in CB activity in these patients. This intriguing

hypothesis is supported by the finding that exogenous II-1 can augment type I cell $[\text{Ca}^{2+}]_i$ and increase chemoafferent frequency (Ackland et al., 2013). Either way, targeting of the CB in early stages of T2D or obesity may prove to be of clinical importance for reducing cardiovascular disease in these large patient cohorts.

6. Conclusion

The CB plays an important role in mediating reflex responses to hypoglycaemia; it contributes to the counter-regulatory release of hepatic glucose reserves and causes a rise in ventilation to match the increase in metabolic rate. The latter acts to preserve arterial CO_2 and blood pH during hypoglycaemic episodes preventing acidosis. Our recent work supports the idea that CB activation during hypoglycaemia is due to the counter-regulatory hormone Adr acting on the type I cell. The finding that Adr can modify CB activity could have further important clinical implications in diseases such as SDB and diabetes where there are chronic rises or fluctuations in plasma Adr concentrations (Figure 2). Whether or not persistent exposure to Adr causes pathological CB remodelling is an interesting area for future research.

Funding

Funding from the BBSRC for E.L.T. during her MRes degree programme is gratefully acknowledged. Z.A PhD studies are sponsored by the Saudi Arabian Cultural Bureau.

References

- Ackland, G.L., Kazymov, V., Marina, N., Singer, M., Gourine, A.V., 2013. Peripheral Neural Detection of Danger-Associated and Pathogen-Associated Molecular Patterns. *Critical care medicine* 41, E85-E92.
- Alvarez-Buylla, R., Alvarez-Buylla, E., Mendoza, H., Montero, S.A., Alvarez-Buylla, A., 1997. Pituitary and adrenals are required for hyperglycemic reflex initiated by stimulation of CBR with cyanide. *Am J Physiol* 272, R392-399.
- Alvarez-Buylla, R., de Alvarez-Buylla, E.R., 1988. Carotid sinus receptors participate in glucose homeostasis. *Respir Physiol* 72, 347-359.
- Atkinson, M.A., Eisenbarth, G.S., Michels, A.W., 2014. Type 1 diabetes. *Lancet* 383, 69-82.
- Balfour, R.H., Hansen, A.M.K., Trapp, S., 2006. Neuronal responses to transient hypoglycaemia in the dorsal vagal complex of the rat brainstem. *J. Physiol.-London* 570, 469-484.
- Bao, G., Metreveli, N., Li, R., Taylor, A., Fletcher, E.C., 1997. Blood pressure response to chronic episodic hypoxia: Role of the sympathetic nervous system. *J. Appl. Physiol.* 83, 95-101.
- Barbosa, T.C., Kaur, J., Holwerda, S.W., Young, C.N., Curry, T.B., Thyfault, J.P., Joyner, M.J., Limberg, J.K., Fadel, P.J., 2018. Insulin increases ventilation during euglycemia in humans. *Am J Physiol Regul Integr Comp Physiol*. doi: 10.1152/ajpregu.00039.2018. [Epub ahead of print] PMID:29590558
- Bartnik, M., Ryden, L., Ferrari, R., Malmberg, K., Pyorala, K., Simoons, M., Standl, E., Soler-Soler, J., Ohrvik, J., Euro Heart Survey, I., 2004. The prevalence of abnormal glucose regulation in patients with coronary artery disease across Europe - The Euro Heart Survey on diabetes and the heart. *Eur. Heart J.* 25, 1880-1890.
- Bell, D.S.H., 2003. Heart failure - The frequent, forgotten, and often fatal complication of diabetes. *Diabetes Care* 26, 2433-2441.

- Bin-Jalilah, I., Maskell, P.D., Kumar, P., 2004. Indirect sensing of insulin-induced hypoglycaemia by the carotid body in the rat. *J Physiol* 556, 255-266.
- Bin-Jalilah, I., Maskell, P.D., Kumar, P., 2005. Carbon dioxide sensitivity during hypoglycaemia-induced, elevated metabolism in the anaesthetized rat. *J Physiol* 563, 883-893.
- Boland, E., Monsod, T., Delucia, M., Brandt, C.A., Fernando, S., Tamborlane, W.V., 2001. Limitations of conventional methods of self-monitoring of blood glucose - Lessons learned from 3 days of continuous glucose sensing in pediatric patients with type 1 diabetes. *Diabetes Care* 24, 1858-1862.
- Borel, A.L., Benhamou, P.Y., Baguet, J.P., Halimi, S., Levy, P., Mallion, J.M., Pepin, J.L., 2010. High prevalence of obstructive sleep apnoea syndrome in a Type 1 diabetic adult population: a pilot study. *Diabetic Medicine* 27, 1329-1329.
- Brown, A.M., Evans, R.D., Black, J., Ransom, B.R., 2012. Schwann cell glycogen selectively supports myelinated axon function. *Ann. Neurol.* 72, 406-418.
- Brown, A.M., Ransom, B.R., 2007. Astrocyte glycogen and brain energy metabolism. *Glia* 55, 1263-1271.
- Brown, A.M., Sickmann, H.M., Fosgerau, K., Lund, T.M., Shousboe, A., Waagepetersen, H.S., Ransom, B.R., 2005. Astrocyte glycogen metabolism is required for neural activity during aglycemia or intense stimulation in mouse white matter. *J. Neurosci. Res.* 79, 74-80.
- Brown, A.M., Tekkok, S.B., Ransom, B.R., 2003. Glycogen regulation and functional role in mouse white matter. *J. Physiol.-London* 549, 501-512.
- Buckler, K.J., Turner, P.J., 2013. Oxygen sensitivity of mitochondrial function in rat arterial chemoreceptor cells. *J Physiol* 591, 3549-3563.
- Carlson, J.T., Hedner, J., Elam, M., Ejnell, H., Sellgren, J., Wallin, B.G., 1993. Augmented resting sympathetic activity in awake patients with obstructive sleep-apnea. *Chest* 103, 1763-1768.

- Cassaglia, P.A., Hermes, S.M., Aicher, S.A., Brooks, V.L., 2011. Insulin acts in the arcuate nucleus to increase lumbar sympathetic nerve activity and baroreflex function in rats. *J. Physiol.-London* 589, 1643-1662.
- Chang, A.J., Ortega, F.E., Riegler, J., Adison, D.V.M., Krasnow, M.A., 2015. Oxygen regulation of breathing through an olfactory receptor activated by lactate. *Nature* 527, 240-244.
- Conde, S.V., Obeso, A., Gonzalez, C., 2007. Low glucose effects on rat carotid body chemoreceptor cells' secretory responses and action potential frequency in the carotid sinus nerve. *J Physiol* 585, 721-730.
- Conde, S.V., Sacramento, J.F., Guarino, M.P., Gonzalez, C., Obeso, A., Diogo, L.N., Monteiro, E.C., Ribeiro, M.J., 2014. Carotid body, insulin, and metabolic diseases: unraveling the links. *Frontiers in physiology* 5, 418.
- Cryer, P.E., Davis, S.N., Shamon, H., 2003. Hypoglycemia in diabetes. *Diabetes Care* 26, 1902-1912.
- Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P., Garg, R., 2005. Metabolic syndrome - A comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation* 111, 1448-1454.
- Dasso, L.L., Buckler, K.J., Vaughan-Jones, R.D., 2000. Interactions between hypoxia and hypercapnic acidosis on calcium signaling in carotid body type I cells. *Am J Physiol Lung Cell Mol Physiol* 279, L36-42.
- Del Rio, R., Andrade, D.C., Lucero, C., Arias, P., Iturriaga, R., 2016. Carotid Body Ablation Abrogates Hypertension and Autonomic Alterations Induced by Intermittent Hypoxia in Rats. *Hypertension (Dallas, Tex. : 1979)* 68, 436-445.
- Del Rio, R., Moya, E.A., Parga, M.J., Madrid, C., Iturriaga, R., 2012. Carotid body inflammation and cardiorespiratory alterations in intermittent hypoxia. *Eur. Resp. J.* 39, 1492-1500.

- Donovan, C.M., Watts, A.G., 2014. Peripheral and central glucose sensing in hypoglycemic detection. *Physiology (Bethesda)* 29, 314-324.
- Duchen, M.R., Biscoe, T.J., 1992a. Mitochondrial function in type I cells isolated from rabbit arterial chemoreceptors. *J Physiol* 450, 13-31.
- Duchen, M.R., Biscoe, T.J., 1992b. Relative mitochondrial membrane potential and $[Ca^{2+}]_i$ in type I cells isolated from the rabbit carotid body. *J Physiol* 450, 33-61.
- Dunn-Meynell, A.A., Routh, V.H., Kang, L., Gaspers, L., Levin, B.E., 2002. Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. *Diabetes* 51, 2056-2065.
- Duran, J., Esnaola, S., Rubio, R., Iztueta, A., 2001. Obstructive sleep apnea-hypopnea and related clinical features in a population-based sample of subjects aged 30 to 70 yr. *Am. J. Respir. Crit. Care Med.* 163, 685-689.
- Elmasry, A., Lindberg, E., Hedner, J., Janson, C., Boman, G., 2002. Obstructive sleep apnoea and urine catecholamines in hypertensive males: a population-based study. *Eur. Resp. J.* 19, 511-517.
- Fitzgerald, R.S., Shirahata, M., Chang, I., Kostuk, E., 2009. The impact of hypoxia and low glucose on the release of acetylcholine and ATP from the incubated cat carotid body. *Brain Res* 1270, 39-44.
- Flemons, W.W., Buysse, D., Redline, S., Pack, A., Strohl, K., Wheatley, J., Young, T., Douglas, N., Levy, P., McNicholas, W., Fleetham, J., White, D., Schmidt-Nowarra, W., Carley, D., Romaniuk, J., Amer Acad Sleep Med Task, F., 1999. Sleep-related breathing disorders in adults: Recommendations for syndrome definition and measurement techniques in clinical research. *Sleep* 22, 667-689.

- Fletcher, E.C., Lesske, J., Behm, R., Miller, C.C., 3rd, Stauss, H., Unger, T., 1992. Carotid chemoreceptors, systemic blood pressure, and chronic episodic hypoxia mimicking sleep apnea. *J Appl Physiol* 72, 1978-1984.
- Folgering, H., Ponte, J., Sadig, T., 1982. Adrenergic mechanisms and chemoreception in the carotid body of the cat and rabbit. *J Physiol* 325, 1-21.
- Gallego-Martin, T., Fernandez-Martinez, S., Rigual, R., Obeso, A., Gonzalez, C., 2012. Effects of low glucose on carotid body chemoreceptor cell activity studied in cultures of intact organs and in dissociated cells. *Am J Physiol Cell Physiol* 302, C1128-1140.
- Gami, A.S., Hodge, D.O., Herges, R.M., Olson, E.J., Nykodym, J., Kara, T., Somers, V.K., 2007. Obstructive sleep apnea, obesity, and the risk of incident atrial fibrillation. *Journal of the American College of Cardiology* 49, 565-571.
- Garcia-Fernandez, M., Ortega-Saenz, P., Castellano, A., Lopez-Barneo, J., 2007. Mechanisms of low-glucose sensitivity in carotid body glomus cells. *Diabetes* 56, 2893-2900.
- Han, S.M., Namkoong, C., Jang, P.G., Park, I.S., Hong, S.W., Katakami, H., Chun, S., Kim, S.W., Park, J.Y., Lee, K.U., Kim, M.S., 2005. Hypothalamic AMP-activated protein kinase mediates counter-regulatory responses to hypoglycaemia in rats. *Diabetologia* 48, 2170-2178.
- Hauton, D., Holmes, A., Ziff, O., Kumar, P., 2013. The impact of acute and chronic catecholamines on respiratory responses to hypoxic stress in the rat. *Pflugers Arch* 465, 209-219.
- Holmes, A.P., Hauton, D., Kumar, P., 2012. The Interaction Between Low Glucose and Hypoxia in the in vitro, Rat Carotid Body. *Adv Exp Med Biol* 758, 123-127.
- Holmes, A.P., Nunes, A.R., Cann, M.J., Kumar, P., (2015). Ecto-5'-Nucleotidase, Adenosine and Transmembrane Adenylyl Cyclase Signalling Regulate Basal Carotid Body Chemoafferent Outflow and Establish the Sensitivity to Hypercapnia, in: Peers, C., Kumar, P., Wyatt, C.N.,

- Gauda, E., Nurse, C.A., Prabhakar, N. (Eds.), *Arterial Chemoreceptors in Physiology and Pathophysiology*. Springer-Verlag Berlin, Berlin, pp. 279-289.
- Holmes, A.P., Ray, C.J., Pearson, S.A., Coney, A.M., Kumar, P., 2017. Ecto-5'-nucleotidase (CD73) regulates peripheral chemoreceptor activity and cardiorespiratory responses to hypoxia. *J Physiol*. doi: 10.1113/JP274498. [Epub ahead of print]. PMID:28560821
- Holmes, A.P., Turner, P.J., Buckler, K.J., Kumar, P., 2016. Moderate inhibition of mitochondrial function augments carotid body hypoxic sensitivity. *Pflugers Arch*. 468, 143-155.
- Holmes, A.P., Turner, P.J., Carter, P., Leadbeater, W., Ray, C.J., Hauton, D., Buckler, K.J., Kumar, P., 2014. Glycogen metabolism protects against metabolic insult to preserve carotid body function during glucose deprivation. *J Physiol* 592, 4493-4506.
- Inzucchi, S.E., Bergenstal, R.M., Buse, J.B., Diamant, M., Ferrannini, E., Nauck, M., Peters, A.L., Tsapas, A., Wender, R., Matthews, D.R., 2012. Management of Hyperglycemia in Type 2 Diabetes: A Patient-Centered Approach. *Diabetes Care* 35, 1364-1379.
- Iwamoto, Y., Ohishi, M., Yuan, M., Tatara, Y., Kato, N., Takeya, Y., Onishi, M., Maekawa, Y., Kamide, K., Rakugi, H., 2011. beta-Adrenergic receptor gene polymorphism is a genetic risk factor for cardiovascular disease: a cohort study with hypertensive patients. *Hypertens. Res.* 34, 573-577.
- Joels, N., White, H., 1968. The contribution of the arterial chemoreceptors to the stimulation of respiration by adrenaline and noradrenaline in the cat. *J Physiol* 197, 1-23.
- Johnson, B.D., Peinado, A.B., Ranadive, S.M., Curry, T.B., Joyner, M.J., 2018. Effects of intravenous low-dose dopamine infusion on glucose regulation during prolonged aerobic exercise. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 314, R49-R57.
- Johnson, J.A., Terra, S.G., 2002. beta-adrenergic receptor polymorphisms: Cardiovascular disease associations and pharmacogenetics. *Pharm. Res.* 19, 1779-1787.

- Kanagala, R., Murali, N.S., Friedman, P.A., Ammash, N.M., Gersh, B.J., Ballman, K.V., Shamsuzzaman, A.S.M., Somers, V.K., 2003. Obstructive sleep apnea and the recurrence of atrial fibrillation. *Circulation* 107, 2589-2594.
- Kelly, A., Dougherty, S., Cucchiara, A., Marcus, C.L., Brooks, L.J., 2010. Catecholamines, Adiponectin, and Insulin Resistance as Measured by HOMA in Children with Obstructive Sleep Apnea. *Sleep* 33, 1185-1191.
- Kim, D., Kim, I., Papreck, J.R., Donnelly, D.F., Carroll, J.L., 2011. Characterization of an ATP-sensitive K(+) channel in rat carotid body glomus cells. *Respir Physiol Neurobiol* 177, 247-255.
- Kim, M.S., Park, J.Y., Namkoong, C., Jang, P.G., Ryu, J.W., Song, H.S., Yun, J.Y., Namgoong, I.S., Ha, J., Park, I.S., Lee, I.K., Viollet, B., Youn, J.H., Lee, H.K., Lee, K.U., 2004. Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat Med* 10, 727-733.
- Koyama, Y., Coker, R.H., Denny, J.C., Lacy, D.B., Jabbour, K., Williams, P.E., Wasserman, D.H., 2001. Role of carotid bodies in control of the neuroendocrine response to exercise. *Am J Physiol Endocrinol Metab* 281, E742-748.
- Koyama, Y., Coker, R.H., Stone, E.E., Lacy, D.B., Jabbour, K., Williams, P.E., Wasserman, D.H., 2000. Evidence that carotid bodies play an important role in glucoregulation in vivo. *Diabetes* 49, 1434-1442.
- Kumar, P., 2007. How sweet it is: sensing low glucose in the carotid body. *J Physiol* 578, 627.
- Lam, S.Y., Liu, Y., Ng, K.M., Lau, C.F., Liong, E.C., Tipoe, G.L., Fung, M.L., 2012. Chronic intermittent hypoxia induces local inflammation of the rat carotid body via functional upregulation of proinflammatory cytokine pathways. *Histochem. Cell Biol.* 137, 303-317.

- Lanza, G.A., Pitocco, D., Navarese, E.P., Sestito, A., Sgueglia, G.A., Manto, A., Infusino, F., Musella, T., Ghirlanda, G., Crea, F., 2007. Association between cardiac autonomic dysfunction and inflammation in type 1 diabetic patients: effect of beta-blockade. *Eur. Heart J.* 28, 814-820.
- Lemus, M., Montero, S., Cadenas, J.L., Lara, J.J., Tejeda-Chavez, H.R., Alvarez-Buylla, R., de Alvarez-Buylla, E.R., 2008. Gaba(B) receptors activation in the NTS blocks the glyceemic responses induced by carotid body receptor stimulation. *Auton. Neurosci-Basic Clin.* 141, 73-82.
- Limberg, J.K., Dube, S., Kuijpers, M., Farni, K.E., Basu, A., Rizza, R.A., Curry, T.B., Basu, R., Joyner, M.J., 2015. Effect of hypoxia on heart rate variability and baroreflex sensitivity during hypoglycemia in type 1 diabetes mellitus. *Clinical Autonomic Research* 25, 243-250.
- Limberg, J.K., Farni, K.E., Taylor, J.L., Dube, S., Basu, A., Basu, R., Wehrwein, E.A., Joyner, M.J., 2014. Autonomic control during acute hypoglycemia in type 1 diabetes mellitus. *Clinical Autonomic Research* 24, 275-283.
- Linton, R.A., Band, D.M., Wolff, C.B., 1992. Carotid chemoreceptor discharge during epinephrine infusion in anesthetized cats. *J Appl Physiol* 73, 2420-2424.
- Lohse, M.J., Engelhardt, S., Danner, S., Bohm, M., 1996. Mechanisms of beta-adrenergic receptor desensitization: From molecular biology to heart failure. *Basic Res. Cardiol.* 91, 29-34.
- Lowry, O.H., Passonneau, J.V., 1964. The Relationships between Substrates and Enzymes of Glycolysis in Brain. *J Biol Chem* 239, 31-42.
- Maahs, D.M., Hokanson, J., Kinney, G.L., Ehrlich, J., Wadwa, P., Garg, S., Snell-Bergeon, J.K., Eckel, R.H., Dabelea, D., Rewers, M.J., 2005. Hypertension prevalence, awareness, treatment, and control in an adult type 1 diabetes population and a comparable general population. *Diabetes Care* 28, 301-306.
- Mahmoud, A.D., Lewis, S., Juricic, L., Udoh, U.A., Hartmann, S., Jansen, M.A., Ogunbayo, O.A., Puggioni, P., Holmes, A.P., Kumar, P., Navarro-Dorado, J., Foretz, M., Viollet, B., Dutia,

M.B., Marshall, I., Evans, A.M., 2016. AMP-activated Protein Kinase Deficiency Blocks the Hypoxic Ventilatory Response and Thus Precipitates Hypoventilation and Apnea. *Am. J. Respir. Crit. Care Med.* 193, 1032-1043.

Manin, G., Pons, A., Baltzinger, P., Moreau, F., Iamandi, C., Wilhelm, J.M., Lenoble, P., Kessler, L., Kessler, R., 2015. Obstructive sleep apnoea in people with Type 1 diabetes: prevalence and association with micro- and macrovascular complications. *Diabetic Medicine* 32, 90-96.

Marrone, O., Riccobono, L., Salvaggio, A., Mirabella, A., Bonanno, A., Bonsignore, M.R., 1993. Catecholamines and blood-pressure in obstructive sleep-apnea syndrome. *Chest* 103, 722-727.

Meglason, M.D., Matschinsky, F.M., 1986. Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab Rev* 2, 163-214.

Montero, S., Lemus, M., Luquin, S., Garcia-Estrada, J., Melnikov, V., Leal, C.A., Portilla-de Buen, E., Roces de Alvarez-Buylla, E., 2014. Nitric oxide in the commissural nucleus tractus solitarii regulates carotid chemoreception hyperglycemic reflex and c-Fos expression. *Nitric Oxide* 36, 87-93.

Murphy, B.A., Fakira, K.A., Song, Z., Beuve, A., Routh, V.H., 2009. AMP-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. *Am J Physiol Cell Physiol* 297, C750-758.

Narkiewicz, K., van de Borne, P.J.H., Montano, N., Dyken, M.E., Phillips, B.G., Somers, V.K., 1998. Contribution of tonic chemoreflex activation to sympathetic activity and blood pressure in patients with obstructive sleep apnea. *Circulation* 97, 943-945.

Nieto, F.J., Young, T.B., Lind, B.K., Shahar, E., Samet, J.M., Redline, S., D'Agostino, R.B., Newman, A.B., Lebowitz, M.D., Pickering, T.G., Sleep Heart Hlth, S., 2000. Association of

sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. *JAMA-J. Am. Med. Assoc.* 283, 1829-1836.

Nishi, K., Stensaas, L.J., 1974. The ultrastructure and source of nerve endings in the carotid body. *Cell Tissue Res* 154, 303-319.

Nunes, A.R., Batuca, J.R., Monteiro, E.C., 2010. Acute hypoxia modifies cAMP levels induced by inhibitors of phosphodiesterase-4 in rat carotid bodies, carotid arteries and superior cervical ganglia. *Br J Pharmacol* 159, 353-361.

Nunes, A.R., Holmes, A.P., Conde, S.V., Gauda, E.B., Monteiro, E.C., 2014. Revisiting cAMP signaling in the carotid body. *Frontiers in physiology* 5, 406.

Nunes, A.R., Holmes, A.P., Sample, V., Kumar, P., Cann, M.J., Monteiro, E.C., Zhang, J., Gauda, E.B., 2013. Bicarbonate-sensitive soluble and transmembrane adenylyl cyclases in peripheral chemoreceptors. *Respir Physiol Neurobiol* 188, 83-93.

Ohshima, Y., Iwase, M., Izumizaki, M., Nakayama, H., Narita, I., Homma, I., 2011. Effects of fasting on hypoxic ventilatory responses and the contribution of histamine H1 receptors in mice. *J Physiol Sci* 61, 73-82.

Ortega-Saenz, P., Pardal, R., Levitsky, K., Villadiego, J., Munoz-Manchado, A.B., Duran, R., Bonilla-Henao, V., Arias-Mayenco, I., Sobrino, V., Ordonez, A., Oliver, M., Toledo-Aral, J.J., Lopez-Barneo, J., 2013. Cellular properties and chemosensory responses of the human carotid body. *J. Physiol.-London* 591, 6157-6173.

Paleczny, B., Siennicka, A., Zacharski, M., Jankowska, E.A., Ponikowska, B., Ponikowski, P., 2016. Increased body fat is associated with potentiation of blood pressure response to hypoxia in healthy men: relations with insulin and leptin. *Clinical Autonomic Research* 26, 107-116.

Pardal, R., Lopez-Barneo, J., 2002. Low glucose-sensing cells in the carotid body. *Nat Neurosci* 5, 197-198.

- Peng, Y.J., Makarenko, V.V., Nanduri, J., Vasavda, C., Raghuraman, G., Yuan, G.X., Gadalla, M.M., Kumar, G.K., Snyder, S.H., Prabhakar, N.R., 2014a. Inherent variations in CO-H₂S-mediated carotid body O₂ sensing mediate hypertension and pulmonary edema. *Proc. Natl. Acad. Sci. U. S. A.* 111, 1174-1179.
- Peng, Y.J., Nanduri, J., Yuan, G., Wang, N., Deneris, E., Pendyala, S., Natarajan, V., Kumar, G.K., Prabhakar, N.R., 2009. NADPH oxidase is required for the sensory plasticity of the carotid body by chronic intermittent hypoxia. *J Neurosci* 29, 4903-4910.
- Peng, Y.J., Yuan, G., Jacono, F.J., Kumar, G.K., Prabhakar, N.R., 2006a. 5-HT evokes sensory long-term facilitation of rodent carotid body via activation of NADPH oxidase. *J Physiol* 576, 289-295.
- Peng, Y.J., Yuan, G., Khan, S., Nanduri, J., Makarenko, V.V., Reddy, V.D., Vasavda, C., Kumar, G.K., Semenza, G.L., Prabhakar, N.R., 2014b. Regulation of hypoxia-inducible factor- α isoforms and redox state by carotid body neural activity in rats. *J Physiol* 592, 3841-3858.
- Peng, Y.J., Yuan, G., Ramakrishnan, D., Sharma, S.D., Bosch-Marce, M., Kumar, G.K., Semenza, G.L., Prabhakar, N.R., 2006b. Heterozygous HIF-1 α deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol* 577, 705-716.
- Peppard, P.E., Young, T., Palta, M., Skatrud, J., 2000. Prospective study of the association between sleep-disordered breathing and hypertension. *N. Engl. J. Med.* 342, 1378-1384.
- Pepper, D.R., Landauer, R.C., Kumar, P., 1995. Postnatal development of CO₂-O₂ interaction in the rat carotid body in vitro. *J Physiol* 485 (Pt 2), 531-541.
- Prabhakar, N.R., Kumar, G.K., Peng, Y.J., 2012. Sympatho-adrenal activation by chronic intermittent hypoxia. *J. Appl. Physiol.* 113, 1304-1310.

Ribeiro, M.J., Sacramento, J.F., Gonzalez, C., Guarino, M.P., Monteiro, E.C., Conde, S.V., 2013. Carotid body denervation prevents the development of insulin resistance and hypertension induced by hypercaloric diets. *Diabetes* 62, 2905-2916.

Rocher, A., Caceres, A.I., Almaraz, L., Gonzalez, C., 2009. EPAC signalling pathways are involved in low PO₂ chemoreception in carotid body chemoreceptor cells. *J Physiol* 587, 4015-4027.

Sacramento, J.F., Ribeiro, M.J., Rodrigues, T., Olea, E., Melo, B.F., Guarino, M.P., Fonseca-Pinto, R., Ferreira, C.R., Coelho, J., Obeso, A., Seica, R., Matafome, P., Conde, S.V., 2017. Functional abolition of carotid body activity restores insulin action and glucose homeostasis in rats: key roles for visceral adipose tissue and the liver. *Diabetologia* 60, 158-168.

Salman, S., Vollmer, C., McClelland, G.B., Nurse, C.A., 2017. Characterization of ectonucleotidase expression in the rat carotid body: regulation by chronic hypoxia. *Am. J. Physiol.-Cell Physiol.* 313, C274-C284.

Schober, A.K., Neurath, M.F., Harsch, I.A., 2011. Prevalence of sleep apnoea in diabetic patients. *Clin. Respir. J.* 5, 165-172.

Shahar, E., Whitney, C.W., Redline, S., Lee, E.T., Newman, A.B., Nieto, F.J., O'Connor, G.T., Boland, L.L., Schwartz, J.E., Samet, J.M., Sleep Heart Hlth Study Res, G., 2001. Sleep-disordered breathing and cardiovascular disease: Cross-sectional results of the sleep heart health study. *Am. J. Respir. Crit. Care Med.* 163, 19-25.

Shin, M.K., Yao, Q.L., Jun, J.C., Bevans-Fonti, S., Yoo, D.Y., Han, W., Mesarwi, O., Richardson, R., Fu, Y.Y., Pasricha, P.J., Schwartz, A.R., Shirahata, M., Polotsky, V.Y., 2014. Carotid body denervation prevents fasting hyperglycemia during chronic intermittent hypoxia. *J. Appl. Physiol.* 117, 765-776.

- Soedamah-Muthu, S.S., Fuller, J.H., Mulnier, H.E., Raleigh, V.S., Lawrenson, R.A., Colhoun, H.M., 2006. High risk of cardiovascular disease in patients with type 1 diabetes in the UK - A cohort study using the General Practice Research Database. *Diabetes Care* 29, 798-804.
- Somers, V.K., Dyken, M.E., Clary, M.P., Abboud, F.M., 1995. Sympathetic neural mechanisms in obstructive sleep-apnea. *J. Clin. Invest.* 96, 1897-1904.
- Stevenson, I.H., Teichtahl, H., Cunnington, D., Ciavarella, S., Gordon, I., Kalman, J.M., 2008. Prevalence of sleep disordered breathing in paroxysmal and persistent atrial fibrillation patients with normal left ventricular function. *European heart journal* 29, 1662-1669.
- Stumvoll, M., Goldstein, B.J., van Haeften, T.W., 2005. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 365, 1333-1346.
- Thompson, E.L., Ray, C.J., Holmes, A.P., Pye, R.L., Wyatt, C.N., Coney, A.M., Kumar, P., 2016. Adrenaline release evokes hyperpnoea and an increase in ventilatory CO₂ sensitivity during hypoglycaemia: a role for the carotid body. *The Journal of physiology* 594, 4439-4452.
- Thorens, B., Mueckler, M., 2010. Glucose transporters in the 21st Century. *Am. J. Physiol.-Endocrinol. Metab.* 298, E141-E145.
- Varas, R., Buckler, K.J., 2006. Regulation of a TASK-like potassium channel in rat carotid body type I cells by ATP. *Adv Exp Med Biol* 580, 167-172; 351-169.
- Vazquez-Nin, G., Costero, I., Aguilar, R., Echeverria, O.M., 1977. Innervation of the carotid body, types of nerve endings and their possible significance. *Acta Anat (Basel)* 98, 233-239.
- Ward, D.S., Voter, W.A., Karan, S., 2007. The effects of hypo- and hyperglycaemia on the hypoxic ventilatory response in humans. *J Physiol* 582, 859-869.
- Wehrwein, E.A., Basu, R., Basu, A., Curry, T.B., Rizza, R.A., Joyner, M.J., 2010. Hyperoxia blunts counterregulation during hypoglycaemia in humans: possible role for the carotid bodies? *J Physiol* 588, 4593-4601.

Wehrwein, E.A., Limberg, J.K., Taylor, J.L., Dube, S., Basu, A., Basu, R., Rizza, R.A., Curry, T.B., Joyner, M.J., 2015. Effect of bilateral carotid body resection on the counterregulatory response to hypoglycaemia in humans. *Exp Physiol* 100, 69-78.

Wellen, K.E., Hotamisligil, G.S., 2005. Inflammation, stress, and diabetes. *J. Clin. Invest.* 115, 1111-1119.

Wyatt, C.N., Mustard, K.J., Pearson, S.A., Dallas, M.L., Atkinson, L., Kumar, P., Peers, C., Hardie, D.G., Evans, A.M., 2007. AMP-activated protein kinase mediates carotid body excitation by hypoxia. *J Biol Chem* 282, 8092-8098.

Xu, F., Xu, J., Tse, F.W., Tse, A., 2006. Adenosine stimulates depolarization and rise in cytoplasmic [Ca²⁺] in type I cells of rat carotid bodies. *Am J Physiol Cell Physiol* 290, C1592-1598.

Yaggi, H.K., Concato, J., Kernan, W.N., Lichtman, J.H., Brass, L.M., Mohsenin, V., 2005. Obstructive sleep apnea as a risk factor for stroke and death. *N. Engl. J. Med.* 353, 2034-2041.

Yaranov, D.M., Smyrlis, A., Usatii, N., Butler, A., Petrini, J.R., Mendez, J., Warshofsky, M.K., 2015. Effect of Obstructive Sleep Apnea on Frequency of Stroke in Patients With Atrial Fibrillation. *Am. J. Cardiol.* 115, 461-465.

Young, T., Palta, M., Dempsey, J., Skatrud, J., Weber, S., Badr, S., 1993. The occurrence of sleep-disordered breathing among middle-aged adults. *N. Engl. J. Med.* 328, 1230-1235.

Young, T., Peppard, P.E., Gottlieb, D.J., 2002. Epidemiology of obstructive sleep apnea - A population health perspective. *Am. J. Respir. Crit. Care Med.* 165, 1217-1239.

Yuan, G., Khan, S.A., Luo, W., Nanduri, J., Semenza, G.L., Prabhakar, N.R., 2011. Hypoxia-inducible factor 1 mediates increased expression of NADPH oxidase-2 in response to intermittent hypoxia. *J Cell Physiol* 226, 2925-2933.

- Yuan, G., Nanduri, J., Khan, S., Semenza, G.L., Prabhakar, N.R., 2008. Induction of HIF-1 α expression by intermittent hypoxia: involvement of NADPH oxidase, Ca²⁺ signaling, prolyl hydroxylases, and mTOR. *J Cell Physiol* 217, 674-685.
- Zhang, M., Buttigieg, J., Nurse, C.A., 2007. Neurotransmitter mechanisms mediating low-glucose signalling in cocultures and fresh tissue slices of rat carotid body. *J Physiol* 578, 735-750.
- Zhang, M., Vollmer, C., Nurse, C.A., 2017. Adenosine and dopamine oppositely modulate a hyperpolarization-activated current I_h in chemosensory neurons of the rat carotid body in co-culture. *J Physiol*. doi: 10.1113/JP274743. [Epub ahead of print]. PMID: 28801916
- Zhou, T., Chien, M.-S., Kaleem, S., Matsunami, H., 2016. Single cell transcriptome analysis of mouse carotid body glomus cells. *J Physiol*. 594, 4225-4251.

Figure Captions

Figure 1. Proposed mechanisms of carotid body stimulation in hypoglycaemia in order to evoke hyperpnoea and preserve arterial PCO_2 and pH. 1) Low glucose is directly sensed by the carotid body type I cell which leads to increased neurotransmitter release. 2) Insulin stimulates insulin receptors on the carotid body type I cell, increasing Ca^{2+} and neurotransmitter secretion. 3) Central sensing of low glucose triggers an increase in sympathetic activity and adrenaline release from the adrenal medulla. Adrenaline acts by augmenting carotid body type I cell CO_2 sensitivity to increase neurotransmitter release. For all three proposals, detailed signalling mechanism(s) are still to be defined. CNS: central nervous system, VCO_2 : CO_2 production.

Figure 2. Potential feedback mechanism for adrenaline in mediating carotid body hyperstimulation and cardiovascular pathology in type 1 diabetes and sleep disordered breathing.

