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Integration of hindbrain and carotid body mechanisms that control the autonomic response to cardiorespiratory and glucoprivic insults

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ABSTRACT

Autonomic reflex responses are critical in restoring changes to circulatory factors reduced beyond the domain of homeostasis. Intermittent hypoxia triggers repeated activation of chemoreflexes, resulting in baroreflex dysfunction and widespread changes in cellular and neuronal activity regulated by sensory/motor pathways. Hypoglycaemia initiates a rapid neurally-mediated counter-regulatory response. This counter-regulatory response to hypoglycaemia increases plasma adrenaline levels, liver glycogenolysis, and thus blood glucose levels. Context-dependent activation of rostral ventral medullary neurons initiates baroreceptor unloading, peripheral chemoreflex firing and the counter-regulatory response to hypoglycaemia. In this review, we briefly focus on the functional integration between peripheral and medullary pathways comprising the sympathetic baroreflex, chemoreflexes, and the counter-regulatory response to hypoglycaemia.

1. Introduction

Autonomic reflex mechanisms are unconsciously activated by multiple neural pathways to maintain homeostasis in response to stressors. This rapid response serves as the first line of defence to counteract external insults and protect the homeostatic internal milieu. Dysregulation of the autonomic nervous system can be difficult to recognise until it manifests in the form of a disease or syndrome. Neurons of the rostral ventrolateral medulla (RVLM) can be classified as catecholaminergic (C1) or non-catecholaminergic (non-C1) (Guyenet, 2006). Broadly speaking, both subsets are implicated in cardiorespiratory and glycaemic regulation (Madden et al., 2006; Ritter et al., 2001). A highly specific organisation exists within medullary structures that respond to cardiorespiratory and glucoprivic changes. The focus in this review will be on the functional integration between peripheral and RVLM pathways comprising the sympathetic baroreflex, chemoreflexes, and the counter-regulatory response to hypoglycaemia.

2. Catecholaminergic systems in the hindbrain

Hökfelt et al. (1974) presented seminal morphological data identifying "adrenaline neurons" that contain phenylethanolamine-*N*-methyltransferase (PNMT), the enzyme required for conversion of noradrenaline to adrenaline. These adrenaline-synthesising cells, termed the C1 neurons, exist in the ventrolateral medulla (bregma level -11.2 to -13.6) (Guyenet et al., 2013). The C1 neurons form the largest group of adrenaline-synthesising neurons in the ventrolateral medulla, with C2 and C3 groups comprising fewer than 30% of the total PNMT-immunoreactive neurons in the rat medulla (Minson et al., 1990). Functionally distinct neurons capable of synthesising both dopamine and noradrenaline include the A1, A2, A5, A6 and A7 subgroups (Bucci et al., 2017). Findings over the past few decades indicate that the C1 neurons not only contain immunoreactivity for tyrosine hydroxylase (TH) and PNMT, but also contain mRNA for various co-transmitters including neuropeptide Y (Blessing et al., 1987; Stornetta et al., 1999), pituitary adenylate cyclase activating polypeptide (PACAP)(Farnham

Abbreviations: AIH, acute intermittent hypoxia; AngII, angiotensin 2; ARC, arcuate nucleus; AT1, angiotensin receptor 1; ATP, adenosine triphosphate; CIH, chronic intermittent hypoxia; CSN, carotid sinus nerve; CVLM, caudal ventrolateral medulla; GABA, gamma aminobutyric acid; HR, heart rate; HAAF, hypoglycaemia-associated autonomic failure; LTF, long-term facilitation; MAP, mean arterial pressure; mRNA, messenger ribonucleic acid; nNOS, neuronal nitric oxide synthase; NTS, nucleus of the solitary tract; OSA, obstructive sleep apnoea; PACAP, pituitary adenylate cyclase activating polypeptide; PNMT, phenylethanolamine-*N*-methyltransferase; pSer40TH, phosphorylated tyrosine hydroxylase; PVN, paraventricular nucleus of the hypothalamus; ROS, reactive oxygen species; RVLM, rostral ventrolateral medulla; TH, tyrosine hydroxylase; 2-DG, 2-deoxy-D-glucose; VMH, ventromedial nucleus of the hypothalamus

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et al., 2008), and enkephalin (Stornetta et al., 2001). Despite the ability of C1 neurons to synthesise catecholamines, there is no direct evidence to support the idea that these neurons use neuropeptides, adrenaline or noradrenaline in cell-to-cell communication. Instead, most RVLM C1 presympathetic neurons contain vesicular glutamate transporter 2 mRNA, and presumably use glutamate as a primary neurotransmitter (Abbott et al., 2014b; Stornetta et al., 2002).

3. Sympathetic-respiratory coupling

Rhythmicity of sympathetic nerve activity is controlled by the central respiratory pattern generator together with baroreceptor inputs and has effects on multiple end-organ outputs. Neurons in the vasomotor and respiratory centres work in synchrony to optimise cardiorespiratory function (Miyawaki et al., 1995). Early studies indicated that in rabbits, sympathetic vasoconstrictor fibres increased their discharge in response to asphyxia (Adrian et al., 1932). Since then, much interest lies in better understanding the mechanics governing cardiorespiratory neuroscience. The existence of separate barosensitive inspiratory and expiratory-related neurons adds to the complex circuitry underpinning respiratory modulation (Miyawaki et al., 1995). Presympathetic C1 and non-C1 neurons in the RVLM display respiratory-modulated firing (Moraes et al., 2013). Enhanced respiratory drive following hypoxia or hypercapnia contributes to activation of the respiratory pattern generator, a nucleus containing motoneurons controlling respiratory muscles. In turn, downstream sympathetic vasomotor activity is affected. One lingering problem includes determining the extent to which C1 neurons contribute to this type of sympathetic-respiratory modulation. A large body of studies incorporating electrophysiological, genetic and molecular techniques have attempted to clarify the role of C1 neurons in sympathetic-respiratory coupling underlying chronic disease states such as neurogenic hypertension.

The chronic intermittent hypoxia (CIH) model is a long-term, extensive, and widely used model to study respiratory-induced neurogenic hypertension (Moraes et al., 2012; Zoccal et al., 2008). Repeated bouts of hypoxia and hypercapnia in varying concentrations increases respiratory drive, heart rate (HR), mean arterial pressure (MAP), efferent sympathetic outflow, and the respiratory-coupled oscillations in blood pressure waveforms known as Traube-Hering waves (see Dick et al., 2014 for in-depth review). Numerous gain and loss-of-function experiments suggest C1 neuronal activation is required to re-capitulate these hallmark features of neurogenic hypertension (Abbott et al., 2013, 2012; Abbott et al., 2009b; Wenker et al., 2017). However, in both in vivo and in situ preparations, the enhanced firing frequency of expiratory-modulated sympathetic nerve activity was not dependent on presympathetic RVLM C1 neuronal activation (Moraes et al., 2017). These studies commonly oppose the notion that over-activation of C1 neurons alone leads to CIH-induced hypertension (Moraes et al., 2017). It remains possible that expiratory-modulation occurs via a group of non-C1 RVLM respiratory-modulated neurons, and both the C1 and non-C1 RVLM neurons contribute to and maintain neurogenic hypertension following CIH (Moraes et al., 2013, 2017). Enhanced respiratory drive appears to induce a form of neural plasticity in RVLM neurons, leading to hyper-sensitisation of the sympathetic-respiratory response. Throughout this process, the co-ordinated post-synaptic release of various neuropeptides may play a critical role in shaping cardiovascular disease outcomes (Pilowsky et al., 1996; Pilowsky and Goodchild, 2002).

4. Hypoxia, hypercapnia and sympathetic elevation

Patients with obstructive sleep apnoea (OSA) suffer from sleep-disordered breathing in the form of recurrent airway blockage. Individuals with OSA are at increased risk of experiencing prolonged sympathetic activation that may evolve to full-blown neurogenic hypertension (Javaheri et al., 2017; Narkiewicz et al., 1998; Peppard et al., 2000; Somers et al., 1988). The mechanisms underlying heightened peripheral sympathetic activity remain uncertain, but considerable research is dedicated to identifying the central autonomic mechanisms that are activated following hypoxia and/or hypercapnia (Dick et al., 2007; Prabhakar et al., 2007; Xing et al., 2012).

Metabolic homeostasis is achieved through maintenance of both arterial and brain pH, O_2 and CO_2 levels. Reductions in arterial O_2 are sensed by chemosensitive carotid body glomus cells, whereas changes in parenchymal pH/CO_2 are detected by specialised pH-sensing neurons located in the retrotrapezoid nucleus (Guyenet et al., 2016; Marina et al., 2018). Additionally, astrocytes, which are specialised glial cells in the central nervous system, contain Ca^{2+} dependent mitochondrial hypoxia sensors (Angelova et al., 2015). Astrocytes contribute to parenchymal pH sensing by increasing astroglial Ca^{2+} influx and adenosine triphosphate (ATP) release (Gourine et al., 2010).

To understand the development of cardiorespiratory dysfunction, it is necessary to appreciate the integration between carotid body chemosensors, astrocytes, and brainstem chemoreceptive cells critical for eliciting the sympathetic response to hypoxia. Peng et al (2003) first demonstrated sensory long-term facilitation (LTF) in the carotid body of CIH-conditioned rats (9 × 15 s of 5% O_2 with 5 min. 21% O_2 in between for 8 h per day) that were subsequently exposed to acute intermittent hypoxia (AIH; 10 × 15 s of 12% O_2 separated by 5 min. under 95% O_2 conditions, hyperoxia). Although AIH alone does not cause sensory LTF in the carotid body, AIH interspersed with hypercapnia (10 x 1 min bouts of PO_2 = 40 Torr, PCO_2 = 60 Torr), and AIH in naïve rats exposed to a hypercapnic background (10 x 1 min bouts of hypoxia [PO_2 = 60 Torr] separated by 5 min. of normoxia [PO_2 = 100 Torr] with a hypercapnic background [PCO_2 50 Torr]) also resulted in carotid body sensory LTF (Roy et al., 2017). Interplay between central and peripheral chemoreceptors appears to accelerate the onset of sympathetic elevation. Brainstem parenchymal O_2 levels are significantly lower under physiological conditions in spontaneously hypertensive rats compared to control (Marina et al., 2015). In these animals, RVLM C1 neurons are surrounded by increased levels of l-lactate and ATP, which may be released by astrocytes during hypoxia via a mitochondrial sensory mechanism (Angelova et al., 2015). The ATP released following astrocytic sensing of hypoxia may further increase the activation of presympathetic RVLM neurons and drive the tonic sympathoexcitation and sustained increases in mean arterial blood pressure. The question of whether chronic brainstem hypoxia can result in the development of hypertension remains unanswered. This experiment would be valuable in assisting our knowledge on whether the overactivity of sympathetic neurons using local brainstem hypoxia can generate disease states such as hypertension.

5. RVLM pathways generating sympathetic outflow following hypoxia

Autonomic reflexes comprise a series of regulatory responses following changes induced by a challenge to an organism. Notable stimuli include rapid increase or decreases in blood pressure (baroreceptor activation or silencing) (Pilowsky and Goodchild, 2002), reductions in blood glucose levels (glucose counter-regulatory response) (Verberne et al., 2016), and reductions in arterial O_2 and/or increased CO_2 levels (peripheral/central chemoreflexes) (Guyenet, 2000; Prabhakar, 2006). Impairments in autonomic reflex function are present in certain diseases and conditions including OSA, 'hypoglycaemia-associated autonomic failure' (HAAF- see later section), and hypertension.

Neurons located in the RVLM are critical for the reflex control of cardiorespiratory and glycaemic homeostasis. In particular, the C1 neurons of the RVLM act as a central locus in co-ordination of cardiorespiratory, stress and metabolic responses. Hypothalamic and reticulospinal neurons control the sympathetic response to hypoxia. Hypoxia is sensed and integrated by the carotid body, a small collection of cells located at the carotid artery bifurcation and exposed to fast

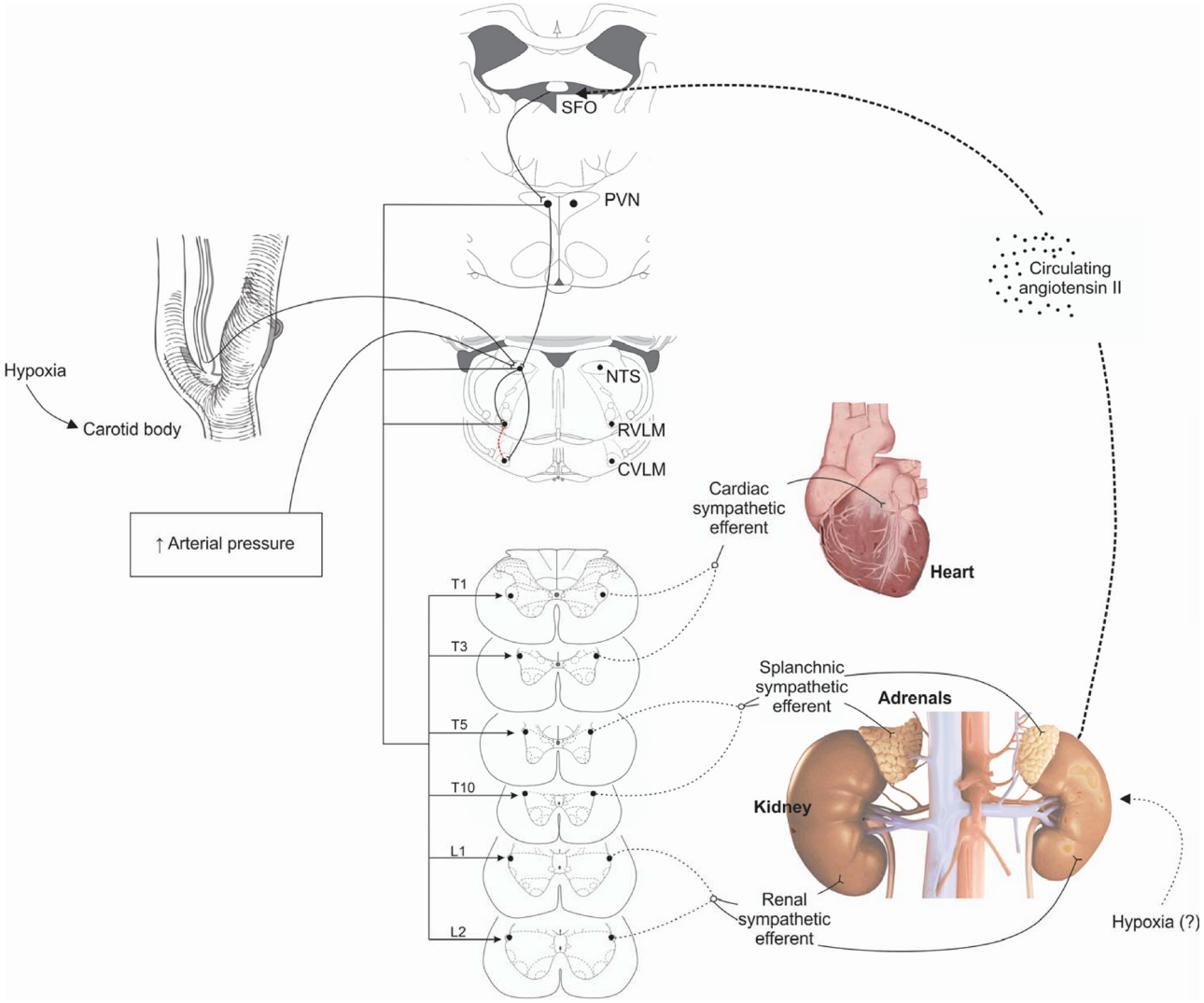

Fig. 1. Schematic depicting sympathetic baroreflex and peripheral chemoreflex activation.
Hypoxia activates carotid body chemoreceptive cells, resulting in increased afferent CSN activity, terminating on neurons in the caudal NTS. A series of possible chemoreflex pathways arise from the NTS to the spinal cord. Consequently, sympathetic outflow through cardiac, splanchnic, and renal nerves is increased. This results in tachycardia, postganglionic innervation of adrenal chromaffin cells and the release of renin from the kidneys. Hypoxia may also directly cause release of renin from the kidneys. Circulating AngII acting through nuclei in the brain with a deficient BBB. Prolonged exposure to intermittent hypoxia sensitises carotid body sensory activity, amplifying these outcomes. Cardiorespiratory dysregulation may eventually manifest as renal artery stenosis and hypertension. Also contributing to cardiorespiratory dysfunction is attenuation of the sympathetic baroreflex. Increased MAP sensed via arterial stretch receptors results in the depolarisation of glossopharyngeal nerve afferents terminating on neurons in the NTS. Baroreflex loading serves to reduce vessel tone, thereby reducing MAP. Glutamatergic inputs from the NTS project to the CVLM, causing activation of inhibitory GABAergic neurons in this region. This results in increased inhibition of RVLM neurons (red), thereby reducing efferent sympathetic tone.
AngII = angiotensin 2, BBB = blood-brain barrier, CSN = carotid sinus nerve, CVLM = caudal ventrolateral medulla, MAP = mean arterial pressure, NTS = nucleus of the solitary tract, RVLM = rostral ventrolateral medulla.

flowing levels of arterial blood. This afferent information is relayed through the carotid sinus nerve (CSN), projecting to first order neurons in the commissural nucleus of the solitary tract (NTS) (Fig. 1). The primary nuclei innervated by the NTS following hypoxia include the RVLM and the paraventricular nucleus of the hypothalamus (PVN) (Coote et al., 1998; Koshiya and Guyenet, 1996). Activation of the PVN using the NMDA-receptor agonist homocysteic acid increased sympathetic discharge in cardiac, splanchnic, and adrenal nerve beds (Coote et al., 1998). In contrast, antagonism of neurotransmission in the PVN using the gamma aminobutyric acid (GABA) agonist muscimol, or the local anaesthetic, lidocaine, attenuated the vasomotor, sympathetic and respiratory effects of peripheral chemoreceptor activation (Reddy et al., 2005). The respiratory and cardiovascular response to hypercapnia (central chemoreflex activation) was not attenuated following injection of muscimol in the PVN, indicating this nucleus is mostly responsible for actioning peripheral, but not central, chemoreflex pathways (Cruz et al., 2008; Reddy et al., 2005).

Hypothalamic PVN neurons give rise to second and third-order pathways innervating the presympathetic RVLM neurons or sympathetic preganglionic neurons in the spinal cord. These pathways include PVN-RVLM (glutamatergic), PVN-RVLM-spinal cord and PVN-spinal cord routes (Coote et al., 1998). Recently, a nNOS-negative population of PVN neurons containing arginine vasopressin was found to be the phenotype activated after exposure to 10% O_2 for 2 h (Coldren et al., 2017). This group also found that presympathetic neurons are not activated in response to hypoxia, 'presympathetic' being defined by retrograde tracing as PVN neurons receiving innervation from sympathetic preganglionic neurons in the spinal cord or RVLM. These differences

may be accounted for by comparing the effects of chemical chemoreflex stimulation (using cyanide) with the more physiologically relevant low O_2 gas supplements (Cruz et al., 2008; Olivan et al., 2001; Reddy et al., 2005). Thus, depending on stimulus intensity, PVN presympathetic neurons may not play an essential role in the hypoxia response, and direct NTS-RVLM connections may supply the important sympathoexcitatory chemoreflex signals.

Neurons in the RVLM certainly play an instrumental role in mediating sympathoexcitation following chemoreflex activation. Peripheral chemoreceptor afferents travel via the CSN, terminating in the caudal NTS, and monosynaptic connections then relay sensory inputs to RVLM neurons. Microinjection of glutamate into the lateral commissural NTS of conscious rats increases MAP and HR (Mauad and Machado, 1998). This increase in MAP was blocked using the α_1 adrenoceptor antagonist prazosin, indicating the pressor effect of NTS chemical stimulation is mediated predominately through the RVLM and downstream α_1 adrenergic receptors (Colombari et al., 1994). Thus, long-term hyper-excitability of CSN inputs to the NTS may contribute to the progression and development of essential hypertension.

There is a heterogenous population of presympathetic C1 and non-C1 neurons in the RVLM that may contribute to sympathetic elevation following a single or prolonged respiratory response (e.g. during chronic intermittent hypoxia-CIH) (Moraes et al., 2013). Optogenetic activation of RVLM C1 neurons causes small but significant increases in MAP, sympathetic nerve discharge, and respiratory frequency (Abbott et al., 2014a, b). Additionally, RVLM C1 neurons rely on glutamatergic neurotransmission to increase respiratory frequency following hypoxia (Abbott et al., 2014a). Below, we will briefly focus on intermittent hypoxia and pathways reported leading to development of increased sympathetic nerve activity in AIH and CIH.

5.1. Acute intermittent hypoxia

Likened to neuronal plasticity, sympathetic LTF is a long-lasting effect in the absence of any stimulus that is observed 60 min. post AIH (Pilowsky et al., 2009; Xing and Pilowsky, 2010). Although initial studies indicated a causative role for increased central respiratory drive in the development of sympathetic elevation (Dick et al., 2007), this was not supported by later studies (Xing and Pilowsky, 2010). Repetitive stimulation of the CSN, or exposure to AIH, are independent stimuli both causing sympathetic and respiratory activity to remain elevated after 60–90 min s (Dick et al., 2007; Kakall et al., 2018; Kim et al., 2016, 2018; Millhorn et al., 1980; Xing et al., 2012; Xing and Pilowsky, 2010). AIH (10% O_2 for 45 s, separated by 5 min. and repeated 10 times) results in activation of RVLM C1 neurons (Fig. 2). This was determined anatomically by immunohistochemistry using specific monoclonal antibodies for TH and phosphorylated TH (pSer40TH) (Nedoboy et al., 2016). Recently, we published data implicating the activation of RVLM PACAP and kynurenate receptors in the development of sympathoexcitation (Kakall et al., 2018). Although targeting specific neuropeptide receptors preceding a physiological insult such as intermittent hypoxia is useful (Xing et al., 2012), it does not allow us to definitively identify which neuronal subset they are released from (C1 or non C1), where these neuropeptides are released from, or where they act (pre- or post-synaptically). In addition to sympathetic control, AIH increases inspiratory-related motor output via a serotonin-dependent pathway (Bautista et al., 2012; Xing et al., 2013), and hypoglossal nerve facilitation (Fuller, 2005). Thus, the AIH model provides a basis for studying the fundamental pathways involved in cardiorespiratory sympathetic regulation and provides the opportunity for manipulations/interventions at an early timescale (~2–3 h).

5.2. Chronic intermittent hypoxia

RVLM C1 neurons in rodents that are exposed to CIH fire at a higher frequency compared to control animals (Moraes et al., 2017). Baseline CSN discharge and sympathoexcitation after hypoxia are increased in rodents exposed to CIH with intact carotid bodies. Additionally, carotid body denervation in rats prevented arterial pressure from increasing after exposure to CIH for ~35 days (Fletcher et al., 1992; Del Rio et al., 2016). Peng et al. (2003) characterised the development of sensory LTF in the carotid body and showed that the altered state of carotid body activity occurs independent of any changes in blood pressure and blood gases. Increased CSN activity was evident after only 3 days of intermittent hypoxia, whereas intermittent hypercapnia did not elicit the same response (Peng et al., 2003). These findings suggest the intermittent activation of peripheral chemoreceptors, rather than central chemoreceptors, following hypoxia is necessary to induce CSN sensory LTF.

The cellular mechanisms causing increased CSN sensory activity following CIH include angiotensin 2 (AngII) mediated signalling through AT1 receptors, and the NADPH-oxidase signalling pathway (Marcus et al., 2010; Schultz et al., 2007). These pathways contribute to the worsened cardiorespiratory reflexes including chemoreflex sensitisation and attenuated baroreflex sensitivity. Sympathetic LTF and the consequent development of hypertension may depend on a DNA methylation “switch”, which prevents the formation of anti-oxidant enzymes and increases levels of reactive oxygen species (ROS) in the carotid body and adrenal medulla (Nanduri et al., 2017). These factors may cumulatively lead to the long-lasting activation of carotid body

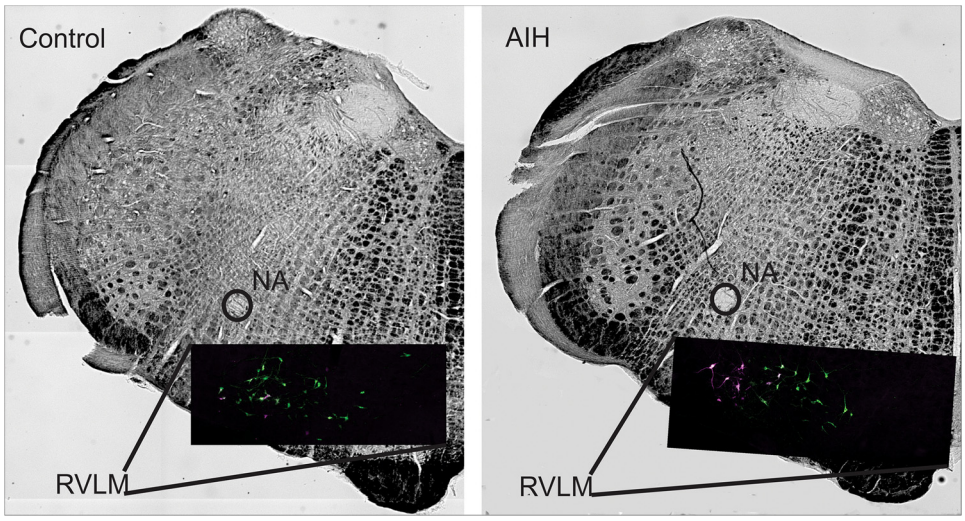

Fig. 2. Intermittent-hypoxia induced activation of catecholaminergic neurons in the RVLM. Anatomical representation indicating changes in the activation state of catecholaminergic neurons in the RVLM after intermittent hypoxia (unpublished). Immunohistochemistry was performed using TH (green) and pSer40TH (purple) antibodies. Catecholaminergic neuronal “activation” is indicated by concomitant staining of both TH and pSer40TH. Acute intermittent hypoxia (10 bouts of 10% O_2 for 45 s with 5 min intervals) caused phosphorylation of catecholaminergic neurons in the RVLM. Brightfield and fluorescence images for Fig. 2 were collected using a Zeiss Axio Imager Z2. *pSer40TH = tyrosine hydroxylase phosphorylated at the serine 40 site, TH = tyrosine hydroxylase.*

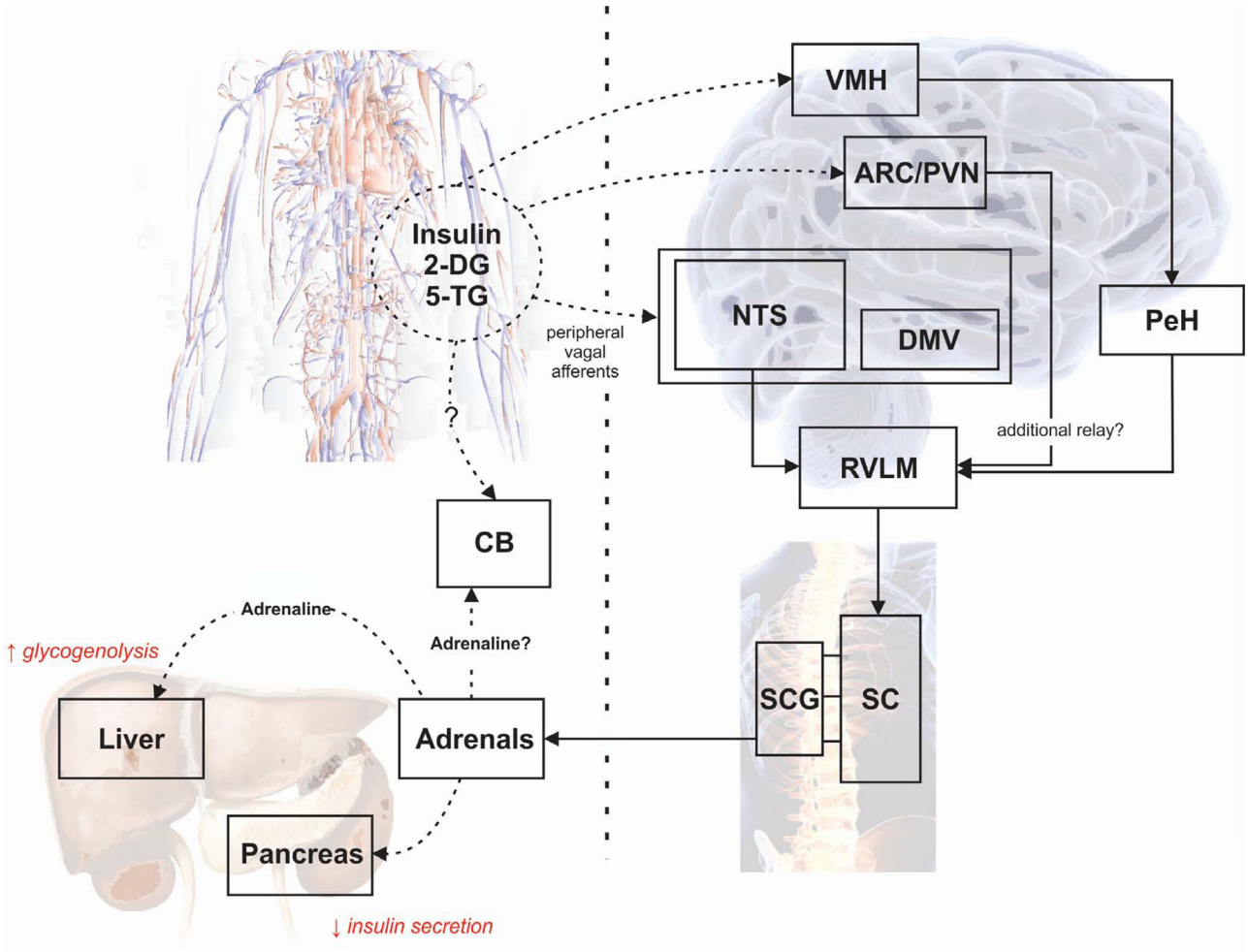

Fig. 3. Neural pathways increasing sympathoadrenal activation following hypoglycaemia/glucoprivation (adapted with permission from Verberne et al. (2016)). Central VMH neurons activated following hypoglycaemia or glucoprivation are inhibitory (GABAergic) and appear to cause disinhibition of PeH orexin-containing neurons. Immediately following this, activation of presympathetic neurons by orexin and glutamate sends excitatory efferent drive through the spinal cord and towards the adrenaline-secreting chromaffin cells. This sympathoadrenal reflex serves to increase plasma adrenaline levels and boost liver glycogenolysis. It remains unknown whether the carotid body acts as an additional peripheral glucose sensor in response to rapid hypoglycaemia and glucoprivation.
ARC = arcuate nucleus, CB = carotid body, DMV = dorsal motor nucleus of the vagus, 5-TG = 5-thioglucose, GABA = gamma aminobutyric acid, NTS = nucleus of the solitary tract, PeH = perifornical hypothalamus, PVN = paraventricular nucleus of the hypothalamus, SC = spinal cord, SCG = sympathetic chain ganglion, 2-DG = 2-deoxy-D-glucose, VMH = ventromedial hypothalamus.

sensory afferent activity. Despite the progression of systemic inflammation indicated by increased plasma thiobarbituric acid levels (lipid peroxidation), carotid body ablation 21 days post-CIH restored the blood pressure, ventilatory response to hypoxia, and cardiovascular baroreflex function in rats (Del Rio et al., 2016).

6. Reflex control of blood pressure and the respiratory contribution

Carotid sinus and aortic arch baroreceptors detect instantaneous changes in peripheral blood pressure. Glossopharyngeal and vagus nerve afferents form a regulatory negative feedback loop to either increase or decrease blood pressure. Tonic baroreflex inhibition is maintained through an additional relay nucleus between the NTS and RVLM, termed the caudal ventrolateral medulla (CVLM). In response to increased blood pressure, activation of inhibitory GABAergic neurons in the CVLM causes inhibition of the RVLM, reducing sympathoexcitatory outflow to the arterioles, and thus counter-balancing the initial increase in blood pressure (Fig. 1). How, then, does interplay between sympathetic baroreflex and chemoreflex activity contribute to cardiorespiratory homeostasis?

Cardiorespiratory dysregulation manifests as sympathetic baroreflex desensitisation and peripheral chemoreflex over-activity (Peng et al., 2012, 2003; Xing and Pilowsky, 2010). These characteristics are found in rodent models of CIH-induced hypertension (Fletcher et al., 1992; Lesske et al., 1997). Furthermore, carotid body chemosensory activity is accentuated in these models, resulting in enhanced vessel constriction beyond recovery by the sympathetic baroreflex. Exactly when, or why, this maladaptive response becomes apparent is unknown. It is important to note that unlike CIH, the AIH protocol does not induce increases in blood pressure; only sympathetic and in some cases phrenic nerve activity (Xing and Pilowsky, 2010). Carotid body hyper-excitability contributes to blood pressure elevation and sympathetic baroreflex dysfunction in rodent models of spontaneous hypertension, renovascular hypertension, and CIH-induced hypertension (Abdala et al., 2012; Peng et al., 2003; Pijacka et al., 2016; Prabhakar et al., 2007). Carotid sinus denervation reduces blood pressure, and improves baroreflex sensitivity and gain, indicating the hyper-responsivity of this peripheral organ is central to over-riding the sympathetic baroreflex in different models of established hypertension (Paton et al., 2013) (Fig. 1).

A subtler connection between baroreflex and chemoreflex

behaviour arises from the activation of receptors belonging to a wide array of neuropeptides. These receptors are typically located pre- or post-synaptically on presympathetic RVLM neurons/fibres, or on sympathetic preganglionic neurons of the spinal cord (Pilowsky and Goodchild, 2002; Pilowsky et al., 2009). It is difficult to trace and identify the root source of neuropeptides activating these specific receptors. However, our group has identified specific neuropeptides with a variety of effects on sympathetic baroreflex (induced by sodium nitroprusside/phenylephrine or aortic depressor nerve stimulation) sensitivity, and peripheral chemosensitivity, using a specialised in vivo preparation consisting of brainstem and/or intrathecal microinjections of pharmacological agonists/antagonists. Neuropeptides serving to improve barosensitivity and reduce chemoreflex sensitivity include catestatin (Gaede et al., 2012; Gaede and Pilowsky, 2010), galanin (Abbott et al., 2009a), and neurotensin (Zogovic and Pilowsky, 2012). Furthermore, activation of muscarinic acetylcholine receptors improves the cardiorespiratory reflexes listed above (Padley et al., 2007). In contrast, RVLM or intrathecal microinjections of PACAP induce a pro-hypertensive phenotype, increasing blood pressure and sympathetic nerve activity, without affecting sympathetic baroreflex sensitivity (Farnham et al., 2012).

7. Reflex control of sympathetic activity following hypoglycaemia or glucoprivation

Insulin promotes glucose uptake and removal from blood resulting in an overall reduction in blood glucose levels. The glucose analogues 2-deoxy-D-glucose (2-DG) or 5-thioglucose inhibit the cellular process of glycolysis, depriving cells of energy and triggering the glucose counter-regulatory response via glucoprivation (Korim et al., 2014, 2016). Both hypoglycaemia (low blood glucose) and glucoprivation (cellular glucose deprivation) initiate a critical reflex response triggering downstream adrenaline release, glycogenolysis and the recovery of normal blood glucose levels. This reflex is dependent on an efferent neural pathway formed by hypothalamic and RVLM neurons (Fig. 3).

Various glucose sensing systems reside in the brain and periphery. Metabolically sensitive glucose-responsive neurons are characterised by the presence of glucokinase and can be either glucose-excited (increased action potential frequency) or glucose-inhibited (decreased action potential frequency) relative to increasing concentrations of extracellular glucose. These glucose sensing neurons are located in the ventromedial nucleus of the hypothalamus (VMH), arcuate nucleus (ARC), perifornical nucleus of the hypothalamus, and the NTS (Fig. 3). The “glucose inhibited” neurons located in the VMH and perifornical nucleus respond to reductions in extracellular glucose and contain nNOS and orexin expression respectively (Chachlaki et al., 2017; Korim et al., 2014).

In addition to peripheral sensors such as the hepatic portal vein and pancreatic β-cell glucose sensors, the carotid body may also possess the ability to sense metabolic changes affecting blood glucose levels. Multiple sensors that integrate blood glucose levels throughout the body are necessary for the maintenance of a healthy glycaemic range. The relative contribution of each sensor to glucose homeostasis remains unclear due to over-compensation from other sensors. CSN afferents project directly to the NTS following stimulation with hypoxia (Kline et al., 2010). Given that carotid body glomus cells are excited by hypoglycaemia, it would be useful to determine the relative significance of carotid body afferents compared to intrinsically glucose-sensing neurons residing in the NTS. This would clarify the relative mechanistic importance of carotid body glucose sensing in relation to independent glucose-sensing neurons.

7.1. Carotid body glomus cells and hypoglycaemia detection

The role of carotid body glomus cells in detecting changes in blood O_2, pH, and CO_2, is well characterised. More recently, it has been suggested these glomus cells were found to secrete neurotransmitters and hormones when placed in glucose-free medium, indicating that glomus cells possess the ability to sense hypoglycaemia (Pardal and Lopez-Barneo, 2002). This demonstration of glucoprivation-induced membrane transduction by the carotid body was the first reported by Pardal and Lopez-Barneo (2002). It appears that glomus cells may respond directly to reduced blood glucose levels, or they may detect increased levels of glucose counter-regulatory hormones such as adrenaline (Thompson et al., 2016). This poses a further possibility that the carotid bodies may act in a mutually reinforcing manner, causing additional release of adrenaline downstream. Inactivation of the carotid body using hyperoxia blunts the counter-regulatory response to hypoglycaemia in healthy humans (Wehrwein et al., 2010). In particular plasma adrenaline, glucagon, cortisol and growth hormone were significantly reduced during hypoglycaemia. Furthermore, a higher glucose infusion rate was required in the hyperoxia-exposed group exposed to maintain mild hypoglycaemia (blood glucose levels at ~3.3 mM).

In glomus cells isolated from rat carotid bodies, neurotransmitter secretory activity was minimal under room O_2 conditions (PO_2 = 150 mmHg) and gradually increased when glucose levels were less than 2 mM glucose (Pardal and Lopez-Barneo, 2002). Patch-clamped glomus cells exposed to glucose-free media facilitate neurotransmitter secretion, which activates carotid body sensory afferent fibres thereby initiating sympathoadrenal activation (Pardal and Lopez-Barneo, 2002). The mechanisms whereby glomus cell depolarisation occur includes activation of inward Na^+ conductance, increased intracellular Ca^{2+} influx and reduced voltage-gated outward K^+ currents (Garcia-Fernandez et al., 2007; Pardal and Lopez-Barneo, 2002). Type 1 glomus cell depolarisation following hypoglycaemia leads to the vesicular release of ATP and acetylcholine, giving rise to excitatory CSN afferents (Zhang et al., 2007). These results suggest that during arterial hypoxia, type 1 glomus cell sensitivity and the neurotransmitter secretory response to glucose is increased. Conditions which produce enhanced carotid body afferent activity, such as repeated hypoxia, may potentiate this hypoglycaemia counter-regulatory pathway and shift the working range of sympathoadrenal activation to higher levels of blood glucose. This would result in excessive glucose production and may be a contributing factor to the hyperglycaemia and glucose intolerance observed in type 2 diabetes.

7.2. Hypoglycaemia-mediated carotid body neuronal inputs

The canonical neural pathway activated by low brain glucose levels begins with disinhibition of glucose-sensitive neurons residing in the hypothalamus, presumably in the VMH, ARC or perifornical nucleus of the hypothalamus (Korim et al., 2014; Murphy et al., 2009; Verberne et al., 2016). These neurons may exist separately from hypothalamic neurons controlling feeding, because insulin-induced hypoglycaemia does not abrogate feeding behaviour in rats (Sanders et al., 2006). The efferent pathway stimulated after hypoglycaemia consists of activated presympathetic RVLM neurons that result in adrenaline release from adrenal chromaffin cells. These neurons are baroinsensitive, and do not respond to elevated MAP caused by aortic occlusion (Verberne and Sartor, 2010). It remains uncertain whether afferent neuronal inputs from the carotid bodies play a large role in glucose sensing during hypoglycaemia. The significance and relationship between co-existing central glucose sensors (neurons in the hypothalamus and brainstem) and peripheral glucose sensors (glomus cells in the carotid body) remains unexplored (Koyama et al., 2000) (Fig. 3). Is there an afferent pathway that arises via increased CSN discharge following hypoglycaemia independently of central glucose-sensing neurons? To answer this question would involve at the very least a preparation which allows for the determination of plasma glucose and the number of Fos positive neurons in the brainstem of carotid body resected rats, compared to normal, following an episode of hypoglycaemia.

The significance of the carotid body as an extra peripherally located

glucose sensor is its close apposition to the brain. Peripheral glucose sensors are known to participate in long-term regulation of glycaemic control. However, carotid body glucose sensing may instead act as part of the rapid reflex initiated following blood glucose changes on a short timescale. Further studies must be conducted to determine whether there is reduced carotid body glucose sensing in conditions such as HAAF. HAAF is a condition whereby the glucose-counter-regulatory response to hypoglycaemia is attenuated by previous episodes of hypoglycaemia. Antecedent insulin-induced hypoglycaemia, or glucoprivation following either 2-DG or 5-thioglucose, are commonly used to experimentally to model HAAF (Sanders and Ritter, 2000, 2001). Whether carotid body hypoglycaemia sensing is dampened in HAAF remains unknown.

8. Physiological integration between cardiorespiratory and glucoprivic reflexes

Increased sympathoadrenal outflow is critical to initiate the counter-regulatory response to hypoglycaemia and glucoprivation. Furthermore, C1 neurons are required to maintain basal glucose levels and initiate adrenaline secretion following 2-DG induced glucoprivation (Madden et al., 2006). Since AIH and CIH both increase sympathetic nerve activity, and consequently adrenal sympathetic outflow, we tested whether intermittent activation of the carotid body induced a hyperglycaemic response. We performed AIH and recorded blood glucose levels up to 60 min. post-stimulus. In our sodium pentobarbital anaesthetised rodent model, intermittent stimulation of the peripheral chemoreflex using 10% O_2 was not sufficient to generate a hyperglycaemic response (unpublished data). This result leads us to believe that the activation of carotid body glomus cells by hypoglycaemia and glucoprivation may occur in response to changes in circulating blood glucose levels, rather than stimulation of peripheral chemoreceptors via the hypoxia pathway. This idea has also been supported by Verberne et al. (2016). Unfortunately, to date, there is no anatomical data comparing activated RVLM neuronal populations following peripheral chemoreflex activation and glucoprivation. Existing anatomical data indicates the existence of stimulus-specific neuronal activation following response to glucoprivation or baroreceptor unloading (Parker et al., 2017). This suggests the afferent pathways triggered by peripheral chemoreflex and baroreflex activation are indeed separate from the neural inputs generating adrenaline release resulting in elevated glucose levels following hypoglycaemia.

Baroreflex activation selectively blocks the peripheral chemoreflex response to hypoxia, but not hypercapnia, in healthy humans (Somers et al., 1991). In a similarly reciprocal manner, increased peripheral chemoreflex activity blunts sympathetic baroreflex function in patients with chronic heart failure (Despas et al., 2012). Thesefindings suggest an inverse relationship between peripheral chemosensitivity and sympathetic baroreflex sensitivity. Recently, a study conducted by Limberg et al. (2014) in healthy humans demonstrated reduced cardiac baroreflex sensitivity during hypoglycaemia, indicating the excitatory sympathoadrenal activation arising from the carotid bodies may also inhibit the cardiac baroreflex. The mechanism by which this occurs is not known, but a subsequent study indicated that patients with bilateral carotid body resections failed to recover from the reduced HR and MAP response following hypoglycaemia (Limberg et al., 2015). Once again, conditions that cause increased peripheral chemoreceptor sensitivity and afferent neuronal drive appear to dampen baroreflex sensitivity and increase aberrant glucose production through the pathways mentioned above. These multi-faceted responses may contribute to the complex ‘metabolic syndrome’.

9. Conclusions

This review aims to summarise the role of carotid body inputs and RVLM neurons in orchestrating cardiorespiratory and glucoprivation-induced reflexes. The inverse relationship between increased peripheral chemoreflex activity and reduced sympathetic baroreflex activity is well documented in the literature. However, the addition of glucoregulation through glomus cells in the carotid bodies invites the possibility that the carotid body is a critical peripheral sensory organ that may contribute to worsening of the metabolic syndrome. Understanding the relevant pathways involved in each response - and how each subserves a unique function - allows for an appreciation of the intricacies involved in maintaining optimal physiological conditions in the organism. In conclusion, both cardiorespiratory and metabolic demands are co-ordinated through the RVLM. Overactivation of carotid body afferents and failure of RVLM neurons to initiate protective reflexes underpins diseases and conditions such as hypertension, hypoglycaemia-associated autonomic failure. These diseases contribute to a complex collection of genetic and lifestyle disorders known as the metabolic syndrome.

Conflict of interest

None

Acknowledgments

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