Adrenaline increases ventilation via a β-receptor and carotid body-mediated mechanism: a role in the hyperventilation of hypoglycaemia?

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Abstract

A role for the carotid body (CB) in glucoregulation has been proposed but the evidence is conflicting. Hypoglycaemia in vivo induces a CB-dependent hyperventilation, but it is not agreed whether this reflects a direct action of reduced blood glucose on the CB, or an indirect effect of adrenaline. We therefore investigated the effects of adrenaline and hypoglycaemia upon ventilation.

Ventilation ($V_E$) was recorded during infusions of adrenaline or insulin (to induce hypoglycaemia) in anaesthetized male Wistar rats. CB-mediated effects were determined by application of hyperoxia at each dose. This was repeated during propranolol infusion. Hypercapnia was applied at control and at the end of adrenaline or insulin infusion.

Adrenaline and hypoglycaemia evoked increases in $V_E$, without an associated change in $P_aCO_2$. Hyperoxia reduced baseline $V_E$ and offset the ventilatory responses. Propranolol reduced baseline $V_E$ and abolished the hypoglycaemia-mediated ventilatory increase, but an increased $P_aCO_2$ occurred. Both hypoglycaemia and adrenaline increased the hypercapnic ventilatory response, which was blocked by propranolol.

These data suggest that adrenaline may underlie the increased $V_E$ seen in hypoglycaemia via a $\beta$-mediated, $O_2$ independent pathway within the CB. It also suggests that the increased $V_E$ during hypoglycaemia is a hyperpnoea that is appropriate to the increased metabolism.
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1. Introduction

1.1 Ventilation and its control

Ventilation is the volume of air entering the lungs per minute ($V_E$, ml.min$^{-1}$), and is equal to the product of respiratory frequency ($R_f$ – number of breaths per minute) and tidal volume ($V_t$ – mls of air entering lungs with each inspiration). $V_E$ is controlled by three parts of the respiratory system; the controller residing in the CNS, the effector respiratory muscles and the afferent sensory input from chemoreceptors and other receptors. The central controller system is located in 2 regions: the pons and medulla of the brainstem, and the cortex. The brainstem is responsible for automatic breathing via three groups of respiratory neurones, which generate the respiratory rhythm. The cortex allows voluntary control over breathing and can override the central pattern generator of the brainstem up to a point. The respiratory muscles are controlled by these central regions to co-ordinate in the action of breathing. The respiratory sensors detect changes in the chemical environment of the blood/ECF. They provide information to the central controller to make necessary changes in ventilation and receive feedback from the effector muscles. The sensors can be divided into three groups: central chemoreceptors, peripheral chemoreceptors and other (largely mechanical) receptors (West, 2012). The central chemoreceptors are located in the medulla near to the respiratory neurones, and respond to changes in the $[H^+]$ of the ECF surrounding them. A decrease in pH leads to increased $V_E$. The ECF composition is mainly determined by the CSF, which is separated from the blood by the blood brain barrier. Therefore central chemoreceptors cannot respond directly to blood $[H^+]$. However CO$_2$ can diffuse from the cerebral vessels across the barrier, leading to an increase in $[H^+]$ (according to the Henderson-Hasselbach equation). This stimulates the chemoreceptors to induce hyperventilation, decreasing CO$_2$ and therefore $[H^+]$, and correcting pH. In this way, $P_{a}CO_2$ (partial pressure of CO$_2$ in the arterial blood) is the major controller of $V_E$ via its effect on the pH of the CSF, and the central chemoreceptors are the most important respiratory sensors in the breath-by-breath control of $V_E$ (West, 2012).
Peripheral chemoreceptors: The carotid body

The peripheral chemoreceptors are present in two locations; the carotid bodies (CBs) and the aortic bodies. The aortic bodies are present above and below the aortic arch, but their contribution is not as significant as that of the carotid bodies in humans. The CBs are located bilaterally at the bifurcation of the common carotid artery to the internal and external carotid arteries (Kumar and Prabhakar, 2012) (Figure 1).

![Diagram A](image1.png)

**Figure 1:** A - A diagram showing the anatomical location of the carotid body (CB). They are positioned bilaterally at the bifurcation of the common carotid (CC) into the internal (IC) and external carotid (EC) arteries. They are afferently innervated by the glossopharyngeal (IXth cranial) nerve, along which chemodischarge is transmitted to the medulla (NTS), which contains cardiorespiratory controller neurone groups. This allows integration of the chemosensory information from the CB, into the characteristic cardiorespiratory reflex response via autonomic and respiratory motor neurones. Image taken from (Kumar, 2007). B - A diagram showing a closer view of the location of the CB. Here it can be seen that the afferent innervation is actually provided by the carotid sinus nerve, a small branch of the glossopharyngeal. Image accessed at http://journals.cambridge.org.

They receive afferent innervation from the carotid sinus nerve (CSN), a branch of the glossopharyngeal (IXth cranial) nerve, which projects from the medulla, allowing conversion
of chemosensory discharge information to a graded characteristic autonomic and cardiorespiratory reflex (Kumar, 2007).

They are highly vascularised organs with a rich supply of capillaries, and receive up to 15 times the blood flow of the brain, relative to size. The efferent innervations of the CB (by sympathetic fibres of the superior cervical ganglion) are thought to impact upon the chemoafferent output by modulating blood flow and hence the level of stimulus detected. The high blood flow results in a small arterial-venous PO$_2$ (partial pressure of O$_2$) difference in spite of a fairly high metabolic demand, facilitating their response to blood gas status (Kumar, 2007).

The CBs are formed of thousands of type I (glomus) cells arranged in 3-5 cell clusters (glomeruli). A single type II cell, which are fewer in number than type I, associates with each cluster. The whole organ is surrounded by protective connective tissue. It is generally accepted that the type I cells are the chemosensory unit, as they make synaptic connections with afferent CSN fibres, and also possess gap junctions with other type I cells, whilst the type II cells have a supportive role much like glia (Kumar and Prabhakar, 2012).

Stimuli of the CB are decreased P$_a$O$_2$ (hypoxaemia), decreased pH (acidosis) and increased P$_a$CO$_2$ (hypercapnia). Hypoxaemia is the primary stimulus, although it must be recognised that the type I cells will sense tissue PO$_2$, rather than P$_a$O$_2$ directly. This is therefore dependent upon the inspired level of O$_2$, haemoglobin concentration (O$_2$ carrying capacity), the structure of the vasculature and therefore the blood flow reaching the cells, as well as the local metabolic demands and diffusion (West, 2012). As already discussed, the high blood flow of the CB is entirely adequate to meet its O$_2$ consumption, and reasonable microvascular PO$_2$s have been reported (Rumsey, 1991) (Whalen, 1973), therefore a low tissue PO$_2$ cannot explain its sensitivity. This suggests that a cellular mechanism for sensing O$_2$ must exist, which has been shown to lead to type I cell membrane depolarisation and a subsequent Ca$^{2+}$-dependent
The upstream sensing mechanism for this pathway is debated (see review (Kumar and Prabhakar, 2012)). The CB response is completely responsible for the increase in $V_E$ seen in response to hypoxaemia in humans, therefore CB resection (CBR) can lead to a loss of the hypoxic $V_E$ response. The CB will also respond to ischaemic/stagnant hypoxia and histotoxic hypoxia as caused by metabolic poisons (Kumar, 2007).

The response of the CB to $P_aCO_2$ accounts for only a small proportion of the total $V_E$ response to hypercapnia; the majority is mediated via the central chemoreceptors. However the peripheral response is much faster and therefore may be useful in situations of sudden increases in $P_aCO_2$, increasing $V_E$ to restore isocapnia. The CB also responds to a decrease in arterial pH, which can be caused by metabolic or respiratory factors. The reflex increased $V_E$ increases excretion of CO$_2$, helping to correct pH. The amount of CO$_2$ in the blood has significant implications for the pH of the blood, as described by the Henderson-Hasselbach equation. $V_E$ therefore allows a large amount of control over acid-base status, although some control also comes from excretion of bicarbonate by the kidneys. The ratio between bicarbonate and PCO$_2$ must be maintained to hold pH at 7.4, and this relationship is represented by the Davenport diagram. The ratio and therefore pH can be disturbed in 4 ways; an acidosis or alkalosis, caused by either respiratory or metabolic factors. Of relevance to hypercapnia is respiratory acidosis, whereby an increased PCO$_2$ leads to a decreased bicarbonate/PCO$_2$ ratio and therefore a decreased pH (West, 2012). For this reason it has been questioned whether CO$_2$ directly acts on the CB, or if the response is mediated by a change in pH, although CO$_2$ has been shown experimentally to have an independent effect when pH is maintained (Biscoe et al, 1970). As the central and peripheral chemoreceptors both respond to CO$_2$/H$^+$, but are separated by the blood brain barrier, they may actually be sensing different environments and appear to be out of phase with one another; however this interaction ensures a smooth response to hypercapnia (Schwartzstein and Parker, 2006).
Other respiratory receptors also exist, for example pulmonary stretch receptors. These other receptors tend to be stimulated by mechanical and noxious stimuli (West, 2012).

As a decreased PO$_2$ is the main stimulus for increased CB activity, it follows that above normoxic levels of PO$_2$ i.e. hyperoxia, would ablate activity of the CB, although tonic firing has still been reported *in vivo* at P$_a$O$_2$S of 400-600mmHg (Kumar, 2007, Biscoe *et al*, 1970). However, despite this, hyperoxia is often used as an experimental tool, allowing a ‘chemical denervation’ e.g. in humans where one cannot perform a carotid sinus nerve section (CSNX), or in animals where use of CSNX or CBR can be technically difficult and hyperoxia provides the advantage of reversibility.

1.2 Carotid body responses: hypoxia and hypercapnia

The PO$_2$ in inspired air (at sea level barometric pressure) is 150mmHg, which drops to around 100mmHg at the alveoli (P$_a$O$_2$) as determined by the balance between the metabolic demand of the tissues and the level of V$_E$. In a healthy lung, arterial O$_2$ (P$_a$O$_2$) is only slightly different to P$_A$O$_2$, and so this level of O$_2$ is present in the systemic circulation reaching the tissues. This difference is contributed to by shunted blood e.g. bronchial and coronary circulation, which becomes O$_2$ depleted before entering the systemic circulation (West, 2012).

As O$_2$ diffuses to the mitochondria in the tissues, PO$_2$ is lowered further to 25-30mmHg and below. PCO$_2$ in the air is negligible, but P$_A$CO$_2$ (and hence P$_a$CO$_2$) is around 40mmHg. Clearly any change in V$_E$ will affect the alveolar and hence arterial levels of O$_2$ and CO$_2$, for example hypoventilation causes PO$_2$ to fall and PCO$_2$ to rise (explained by the alveolar V$_E$ equation, as metabolic CO$_2$ production will continue). The PO$_2$ of blood can also be affected by the relationship between V$_E$ and perfusion, where a mismatch i.e. perfusion of a poorly ventilated region, can lead to a decreased PO$_2$ and an increased PCO$_2$. Hypoventilation, diffusion limitations, shunt and V$_E$-perfusion mismatch are all physiological causes of
hypoxaemia and can also cause hypercapnia (increased $P_aCO_2$) although this tends to be quickly corrected by an increase in $V_E$ driven by chemoreceptors (West, 2012).

**Hypoxia**

As discussed, hypoxia (low $O_2$ content) or hypoxaemia (low $O_2$ specifically in the blood) is the primary and most powerful stimulus of the CB, and so has received much research attention. As tissue $PO_2$ decreases and tissue becomes hypoxic, the CB type I cells respond by increasing $Ca^{2+}$-dependent neurosecretion leading to a rapid and sustained increase in neural discharge, the frequency of which increases exponentially with the stimulus (reviewed by Kumar and Prabhakar, 2012). An example raw trace showing the increased discharge in the carotid sinus nerve in response to transition from hyperoxia to hypoxia, and back again, as well as superimposed single action potentials, are shown below in Figure 2.

![Figure 2: A raw trace showing in vitro chemodischarge of the CSN. Discharge increased upon decreasing $PO_2$ from hyperoxic to hypoxic levels, which was reversed on return to hyperoxia. Pepper et al (1995).](image)

A gradually increasing discharge is seen between $P_aO_2$s of 400-140mmHg. At around 100mmHg the slope of this response increases, reaching a maximum at ~30mmHg. The transmission of this discharge to the medulla results in a graded cardiorespiratory response including hyperventilation (an increased $V_E$ above metabolic demand, primarily via an
increased $R_i$), which decreases $P_{A\text{CO}_2}$, increasing $P_{A\text{O}_2}$ and hence $P_a\text{O}_2$. Bradycardia, decreased cardiac output, peripheral vasoconstriction and an increased adrenomedullary output can also be triggered, although these can often be hidden by hyperventilation induced feedback responses e.g. by pulmonary stretch receptors (see review Marshall, 1999). In very severe hypoxia, discharge can decrease and the reflex response begins to fail (Kumar, 2007).

**Hypercapnia**

As previously mentioned, the CB can also respond to hypercapnia. The contribution of the CB to this response is generally thought to be small, with most of the response being mediated by central chemoreceptors. However it has been reported that the CBs may account for 30-50% of the hypercapnic ventilatory increase, even with a concurrent hyperoxia (Bruce and Cherniack, 1987, Heeringa *et al*, 1979, Nattie, 1999), although some of this response may be due to the CB reflex response leading to an increased central sensitivity (Blain *et al*, 2010). It has also been shown that dogs with isolated CBs perfused to maintain basal activity, show an increase in the time taken to respond to hypercapnia (Smith *et al*, 2006). Despite this significant contribution by the CB to the response to CO$_2$, the majority of research has focused on the transduction of the O$_2$ signal. In contrast to O$_2$, where basal discharge persists even in hyperoxia, if $P_a\text{CO}_2$ falls below 18-25mmHg this basal discharge is stopped (Bartels *et al*, 1956, Eyzaguirre and Lewin, 1961). Reduction in $P_a\text{CO}_2$ can also abolish discharge induced by hypoxia, although this may just be as a consequence of a non-physiological pH (Kumar and Prabhakar, 2012).

CB chemoafferent discharge increases linearly up to around a $P_a\text{CO}_2$ of 65mmHg, after which it decreases and plateaus (Biscoe *et al*, 1970), however the amplitude of this discharge is smaller than that seen during hypoxia (Fitzgerald and Parks, 1971, Lahiri and Delaney, 1975, Pepper *et al*, 1995), which may again be due to an affect of the decreased pH on the type I cell secretory processes (Rocher *et al*, 2009).
The response to $P_aCO_2$ is faster than that to $P_aO_2$, which suggests that fluctuations in $P_aCO_2$ may cause oscillating discharges in CB activity, presenting a candidate for the transmission of information to the brain during exercise (or situations of increased metabolism) (Band et al, 1978, 1980, Kumar et al, 1988). This may be in association with some other blood-borne mediator such as a hormone (Bin Jaliah et al, 2005, Maskell et al, 2006), or via changes in $[K^+]$, as blood gas tension oscillations alone cannot fully explain $V_E$ changes in exercise and the reliability of signalling in this manner via the blood to the CB is questioned (Nye, 1994).

As described, the three stimuli of the CB are responded to individually, potentially via a range of different sensors and transduction processes, but producing the same cardiorespiratory reflex response. The stimuli can also interact, potentiating this response beyond the simple sum of individual responses (Nielsen and Smith, 1952).

As discussed, the $V_E$ response to a decrease in $P_aO_2$ is mediated solely by peripheral chemoreceptors, and is investigated by inhalation of hypoxic mixtures. Within this mixture PCO$_2$ can be kept constant and under this condition PO$_2$ has to fall to $\sim$50mmHg before a ventilatory response is seen. However if PCO$_2$ is increased in combination with hypoxia, $V_E$ will increase at a PO$_2$ $\sim$100mmHg. This is why CO$_2$ is the major controller of $V_E$, whilst PO$_2$ becomes more important in pathological conditions such as chronic lung disease where CO$_2$ is chronically retained, or at altitude where decreased barometric pressure decreases the partial pressure of gases resulting in chronic hypoxia. $P_aCO_2$ is therefore held within a tight range.

The ventilatory response to CO$_2$ is usually investigated by inhalation of a gas mixture containing CO$_2$ or a re-breathing technique, and in humans it has been shown that $V_E$ increases 2-3L.min$^{-1}$ for each 1mmHg increase in PCO$_2$. As mentioned, most of this response is mediated by the central chemoreceptors, but peripheral chemoreceptors respond more rapidly (West, 2012).
1.3 Glucoregulation

Glucose is the main substrate utilised in metabolism to produce ATP and the only energy source utilised by the brain, and as such there is a requirement for a system by which plasma glucose levels are monitored and maintained within a set range (~4-7mmol/L). This is achieved by the existence of glucose sensitive sites, which are able to induce a neuroendocrine reflex response when stimulated by a change in plasma glucose levels. The main glucose-sensing sites that trigger this counter-regulatory response are peripheral; the pancreatic $\alpha$ and $\beta$-cells (Burcelin et al, 2008), the liver (Donovan et al, 1991), and the hepatic portal vein (Hevener et al, 1997), although some central centres are also sensitive to glucose (Burdakov et al, 2005). In response to hypoglycaemia, insulin secretion is halted to decrease uptake of glucose by e.g. skeletal muscle. Glycogenolysis (the breakdown of glycogen to glucose in the liver and skeletal muscle) and gluconeogenesis (production of glucose from the breakdown of glycerol and glutamine in adipose tissue (lipolysis) and the kidney respectively) can be stimulated to increase appearance of glucose in the blood. These pathways are regulated hormonally, for example catecholamines (adrenaline and noradrenaline) are released in response to low glucose. They can increase the rate of glycogenolysis by action at glucagon releasing $\alpha$-pancreatic cells, and reduce insulin production and release by $\beta$-cells. Glucagon decreases the synthesis, and increases breakdown, of glycogen (the storage form of glucose). Both catecholamines and glucagon can also increase gluconeogenesis and glucose delivery to the blood in exercise or stress. Cortisol is also released in response to hypoglycaemia, and can potentiate the effects of glucagon, as well as increasing gluconeogenesis. Another of these counter-regulatory mediators is growth hormone, which acts to decrease tissue glucose uptake and increase both gluconeogenesis and lipolysis (Yeo and Sawdon, 2007).

Insulin is the major hormone responding to increased blood glucose, and acts to increase expression of glucose transporters (e.g. GLUT-4), increase the synthesis of glycogen, with a
corresponding decrease in glucagon production, decrease in gluconeogenesis and lipolysis and
increase in triglyceride synthesis (another form of glucose storage) (Yeo and Sawdon, 2007).

Figure 3 shows these major hormonal control pathways of plasma glucose:

![Diagram of hormonal control of blood glucose](image.png)

Figure 3: An overview of the hormonal control of plasma glucose levels. Image taken from Yeo and Sawden, (2007).

1.4 Role for the CB in glucoregulation

As well as the above established sites of glucose sensing, there is also a suggested role for the
CB as a glucosensor. Due to the high blood flow and metabolic rate of the CB, it is well suited
for a role in sensing a metabolic substrate such as glucose. However discrepancies in the data
are present between species and preparation types and controversy exists over low glucose as a
direct CB stimulus.

For example, Almaraz *et al* (1984) showed no increase in neural discharge in response to low
superfusate glucose concentration in an isolated cat CB preparation, or even a decrease in
neurotransmitter release/discharge frequency in zero glucose for around 2 hours. After this
time a small increase in activity has been seen, before tissue death (Conde *et al*, 2007).

Another *in vitro* intact cat CB study showed that removal of glucose for 12 mins had no effect
on absolute release of neurotransmitters, also supporting no direct role for low glucose (Fitzgerald et al, 2009).

García-Fernández et al (2007) demonstrated the presence of GLUT-1, GLUT-3 and GLUT-4, but not the GLUT-2 glucose transporter in the rat CB, which has been shown to be essential for glucose sensitive cells via GLUT-2 -/- mice (Thorens, 2001). CB cells have also been shown to express no glucokinase enzyme or specific $K_{\text{ATP}}$ channel subunits that are expressed in liver, pancreatic cells and some central glucose sensitive neurons (Thorens, 2001). The fact that the CB does not possess these characteristics typical of other glucosensors, coupled with the discussed in vitro data, disagrees with any role for the CB in sensing low glucose directly.

However there is also substantial evidence for a direct action of low glucose on the CB. In vitro studies have shown that low and zero glucose could elicit a dose-dependent increase in neurotransmitter secretion in rat and mouse thin CB slice preparations (Pardal & Lopez-Barneo, 2002, Lopez-Barneo, 2003, Garcia-Fernandez, 2007), and co-cultures of rat CB type I cells (Zhang et al, 2007).

An in vivo infusion of glucose into the vascularly isolated cat CB sinus reduced activity by 20% and increased the threshold for the hypoxic response (Alvarez-Bullya, 1988). This group also showed that direct CB stimulation by sodium cyanide (a metabolic inhibitor) increased glucose output from the liver and increased brain retention of glucose, via an adrenal and sinus nerve mediated mechanism (Alvarez-Bullya, 1988, 1997). Intracarotid injections of glucose have also been shown to reduce the normal counter-regulatory response to hypoglycaemia in dogs (Frizell, 1993).

More recent in vivo studies have utilised the hyperinsulinaemic hypoglycaemic clamp protocol to investigate the role of the CB in glucose regulation. Koyama et al (2000) performed hypo- and euglycaemic clamps in conscious dogs. They showed that an increased glucose infusion was required to clamp plasma glucose after CBR (Figure 4) and that glucagon and cortisol counter-
regulatory responses were significantly reduced. The adrenaline response was also reduced by 50%, but not significantly.

They used the same methods to evaluate the role of the CB in maintaining plasma glucose during exercise (Koyama et al, 2001), and saw a mismatch between glucose production and disappearance at the onset of exercise, as well as blunted glucagon and noradrenaline counter-regulatory responses in CBR animals. Although the authors suggested low glucose as the direct stimulus, the effect of the counter-regulatory hormones cannot be discounted.

As hinted at previously, the CB possesses polymodality, whereby any of the stimuli to increase chemoafferent discharge results in the same set of cardiorespiratory reflexes. Based on this, hyperinsulinaemic hypoglycaemia, if acting at the CB, would be expected to induce a hyperventilation and associated hypocapnia. This was investigated by Bin Jaliah et al (2004) using eu- and hypoglycaemic clamp protocols in anaesthetised rats. An increased $V_E$ was seen in correlation with the decrease in plasma glucose levels in sham animals; whereas a reduced basal $V_E$ and no increased $V_E$ response to hypoglycaemia was seen in CSNX animals. However a corresponding in vitro preparation in the same lab showed no increase in single
fibre discharge in response to low or removed superfusate glucose, suggesting that low glucose was not the direct stimulus (Bin Jaliah, 2004, 2005). Moreover, in vivo, the hyperventilation seen in the sham animals was not accompanied by a hypocapnia, suggesting that the hyperinsulinaemic hypoglycaemia had increased metabolism, resulting in an isocapnic hyperpnoea. This was addressed by a consequent experiment (Bin Jaliah et al., 2005) using the same methods, which showed sham animals had an increased CO₂ chemosensitivity in hypoglycaemia, not seen in the CSNX group. Again a corresponding in vitro preparation showed opposing results, with a low glucose superfusate blunting the response to increased CO₂ (Bin Jaliah, 2005). These experiments suggest a potential role for the CB in matching Vₑ to metabolism, posing a potential explanation for what is seen during exercise (as suggested by Band et al., 1978, 1980, Kumar et al., 1988). Vₑ rises almost immediately, closely matching the increase in O₂ consumption with increased CO₂ excretion, maintaining arterial PCO₂, O₂ and pH.

As no response was seen in vitro, Bin Jaliah et al. (2005) suggested that this increased peripheral CO₂ sensitivity may be mediated hormonally, for example by adrenaline. This is not a new idea, as adrenaline has previously been suggested to play a role in exercise hyperpnoea (Linton et al., 1992), and increased levels of adrenaline are seen in both hypoglycaemia (Vollmer et al., 1997) and exercise (Christensen et al., 1983). Adrenaline, as well as other adrenoreceptor agonists, have previously been shown to increase ventilation in vivo (e.g. Whelan and Young, 1953, Young, 1957, Joels and White, 1968, Heistad et al., 1972, Maskell et al., 2006), an action which could be blocked by CSNX or application of hyperoxia (Joels and White, 1968, Heistad et al., 1972, Maskell et al., 2006) which is consistent with a direct action on the CB, rather than an action via the effect of adrenaline on blood flow. Joels and White (1968) showed that this increase in ventilation was accompanied by an increased chemoreceptor discharge. They also showed that a similar increase in ventilation and discharge could be achieved by infusion directly into the carotid.
artery, and that this could be ablated by CSNX. Heistad et al (1972) and Maskell et al (2006) showed that the β-adrenoreceptor blocker propranolol was able to prevent the observed ventilatory responses, suggesting a potential mechanism.

However in vitro studies have revealed opposing results; adrenaline doses of 10-220µg, applied via the superfusate onto an isolated CB and sinus nerve, produced either no response or a decrease in chemodischarge (Eyzaguirre and Koyano, 1965). These findings have been replicated by co-workers, who saw a dose-dependent inhibition of afferent fibre discharge at doses of 1µM and above, and no effect at lower doses, in a similar CB preparation (unpublished data, Holmes and Kumar, 2011).

Ward et al (2007) carried out hypo and hyperglycaemic clamp experiments in humans and showed that glucagon, cortisol, adrenaline and noradrenaline increased in response to hypoglycaemia (Figure 5), as did basal $V_E$ and the ventilatory response to hypoxia.

![Graphs showing levels of glucagon, adrenaline, noradrenaline and cortisol increased in response to hypoglycaemia.](image)

Figure 5: A series of graphs showing that levels of glucagon, adrenaline, noradrenaline and cortisol were all increased in response to hypoglycaemia. Taken from Ward et al (2007).
This is consistent with an effect of hypoglycaemia on the CB, but again cannot rule out the possibility of mediation via the release of the counter-regulatory hormones rather than low glucose directly. Wehrwein et al (2010) also carried out hypoglycaemic clamps in humans, but under normoxia or hyperoxia to isolate CB involvement. The glucose infusion required to maintain the clamp was 45% higher and the normal counter-regulatory hormonal response was significantly blunted under hyperoxia i.e. without CB input (Figure 6).

Figure 6: A shows the glucose infusion rate required to maintain a plasma glucose clamp was increased in the presence of hyperoxia i.e. when CB function was ablated. G shows that the amount of counter-regulatory hormone release under hyperoxia was reduced compared to normoxic conditions. Taken from Wehrwein et al, (2010).

This supports the hypothesis originally proposed by Koyama et al (2000) that intact CB’s are required for a normal response to hypoglycaemia, but still does not clarify whether low glucose is the stimulus, or whether it could be adrenaline.
2. Aims and Hypotheses

The CB is a peripheral chemoreceptor responding to changes in arterial gas tensions and pH. It has also been proposed to have a role in glucoregulation, although it is unknown whether the direct stimulus is low glucose or some other blood-borne mediator such as a counter-regulatory hormone.

Based on the current evidence, this project aimed to further investigate the role of the CB in glucoregulation, specifically with respect to how the CB is stimulated in hypoglycaemia e.g. directly by low glucose or by the associated counter-regulatory hormone adrenaline. The interaction between hypoglycaemia/adrenaline and the hypercapnic ventilatory response was also investigated.

The hypotheses were as follows:

• Administration of the hypoglycaemic counter-regulatory hormone adrenaline would activate the CB via $\beta$-adrenoreceptors, increasing basal $V_E$. Hyperoxia and propranolol would ablate CB-mediated increases in $V_E$.

• Administration of the counter-regulatory hormone adrenaline would increase hypercapnic sensitivity.

• Basal $V_E$ would increase during an insulin-induced hypoglycaemic ramp. Hyperoxia and propranolol would ablate this CB-mediated increase in $V_E$.

• Hypercapnic sensitivity would be increased during hypoglycaemia.
3. Methods

3.1 Animals

Male Wistar rats were sourced from Charles River Laboratories, with body weights of 260-415g on the day of experimentation. The animals were housed in single sex groups, at a temperature of 21±1°C and humidity of 40-45%, under a 12-hour light/dark cycle (lights on at 7:00am). Food and water was available ad libitum, until food was withdrawn from midnight before experimentation to ensure stable and consistent basal blood glucose levels.

The care of the animals and all procedures performed were carried out by Home Office licensees in line with the Home Office (UK) guidelines, University of Birmingham guidelines on ethical use of animals and the Animals (Scientific Procedures) Act (UK) 1986.

3.2 Anaesthesia and surgery

Anaesthesia was induced with 4% isoflurane in O_{2} at 4 L min^{−1} (Merial Animal Health Ltd.). Surgery was then carried out as follows (see Figure 7): the right jugular vein was isolated and cannulated (ID 0.5mm, OD 1.5mm), allowing maintenance of anaesthesia with a 17-20 mg.kg^{−1}.hour^{−1} infusion of Alfaxan® (Vétoquinol UK Ltd.) using a syringe driver (Perfusor®, securaFT, B. Braun.), and 0.1ml boluses as necessary. The trachea was isolated and cannulated with a stainless steel T-piece cannula. The right brachial artery was isolated from the brachial plexus and cannulated (ID 0.4mm, OD 0.8mm). The right femoral artery and vein were isolated from the femoral sheath and cannulated (ID 0.58mm, OD 0.96mm, and 3 x ID 0.28mm, OD 0.61mm, each soldered to an ID 0.58mm, OD 0.96mm, respectively). The left femoral artery was isolated.

Plane of anaesthesia was monitored throughout by assessing response to a strong paw pinch. Core body temperature was also monitored and maintained at 37°C throughout, via a rectal temperature probe linked to a homeothermic heat pad system (Harvard Apparatus) the animal was positioned upon. All cannulae were filled with heparinised saline (20 Units/ml Heparin,
LEO® Pharma) and sourced from Portex™, Smiths Medical. Suture was from Look®, Surgical Specialities Corporation and needles (0.5x25, 0.6x30mm and 0.8x40mm, Microlance™) and syringes (1, 2, 5 and 10ml, Plastipak™) were from BD.

Figure 7: A diagram showing the surgical set-up used. The jugular vein was cannulated to allow infusion of anaesthetic (Alfaxan, 17-20mg.kg⁻¹.hr⁻¹). The trachea was cannulated to allow airflow to be monitored via a spirometer, allowing respiratory frequency (R_f) and tidal volume (V_t) to be derived. The brachial artery was cannulated to record mean arterial blood pressure (MABP), from which heart rate (HR) was derived. The right femoral artery was cannulated with a specialised line for taking blood samples. The femoral vein was cannulated with 3 cannulae soldered to larger cannulae, to allow multiple drug infusions. The femoral vascular conductance (FVC = FBF/MABP) and ventilation (V_E = R_f x V_t) were also calculated.

### 3.3 Equipment and data

A spirometer (MacLab, ADInstruments) was attached to the tracheal T-piece to measure airflux, allowing calculation of respiratory frequency (R_f), tidal volume (V_t) and minute ventilation (V_E = R_f x V_t). Animals breathed room air, but a plastic tube running from cylinders of N₂, O₂ (Boc) and CO₂ (Murex) could be attached to the spirometer via a t-piece, allowing control of inspired gases by a rotameter (Cole Parmer).

A pressure transducer (Capto) was attached to the brachial cannula to monitor arterial blood pressure (ABP) (via a MacLab Bridge Amp, ADInstruments), from which mean ABP and HR were derived. Blood samples were taken via a specialised femoral artery cannula, which minimised blood loss and ensured a pure sample. The samples were used to monitor blood
glucose (Accu-Chek®, Aviva), blood gases and haematocrit (150µl capillary tubes and Gem® 4000 premier analyser, Instrumentation Laboratory Ltd.). The isolated femoral artery had a flow probe (Transonic Systems Inc.) attached to monitor femoral blood flow (FBF), an index of hindlimb skeletal muscle blood flow, and also allowing calculation of femoral vascular conductance ($FVC = MFBF/MABP$). The cannulae in the femoral vein were used to give drug infusions (see section 3.4) using syringe drivers (kd Scientific). Data was recorded at sampling frequencies of 100-1000 samples/second, using PowerLab and Labchart software (ADInstruments) on a Mac OS X computer. The experimental set-up is shown in Figure 8 below.

Figure 8: A photograph showing the experimental set-up used. A spirometer was attached to the tracheal T-piece to measure airflux, allowing calculation of respiratory frequency ($R_f$), tidal volume ($V_t$) and minute ventilation ($V_E = R_f \times V_t$). A plastic tube provided a gas supply from cylinders of $N_2$, $O_2$ and $CO_2$, which could be attached to the spirometer via a t-piece. A pressure transducer was attached to the brachial cannula to monitor arterial blood pressure (ABP), from which MABP and HR were derived. Blood samples were taken via a specialised cannula in the right femoral artery. The cannulae in the right femoral vein were used to give drug infusions. The isolated left femoral artery had a flow probe attached to monitor femoral blood flow (FBF), also allowing calculation of femoral vascular conductance ($FVC = MFBF/MABP$). Core body temperature was monitored and maintained at 37°C via a rectal temperature probe linked to a homeothermic heat pad system.
3.4 Drugs

Adrenaline (E4250 - Sigma®) and propranolol (P0884 - Sigma®) were dissolved in saline. Insulin (Hypurin® Bovine Neutral, CP Pharmaceuticals Ltd.) was made up in Gelofusine® plasma expander (4% w/v, Dechra Veterinary Products) and infused using a 10ml glass syringe (81601, Hamilton) to minimise adherence to the equipment.

3.5 Experimental protocols

Before any experiment, 40 minutes post-surgery stabilisation was allowed. A 20-minute baseline was then recorded, during which blood glucose was sampled at 0, 10 and 20 minutes, and a blood gas was taken at 20 minutes. Blood glucose and gas samples were then taken periodically throughout each experiment.

3.5.1 - Group 1: The effect of adrenaline on ventilation

The effect of adrenaline on ventilation was investigated (n=6) using infusions at concentrations between 0.1ng–1µg.kg⁻¹.min⁻¹. 5 minutes was allowed for each dose to reach a steady state, before CB-mediated effects were determined with 30 seconds exposure to 100% O₂ (hyperoxia) which resulted in PₐO₂ > 300 mmHg, and was also carried out at baseline.

After recovery from adrenaline, a 0.3mg.kg⁻¹.min⁻¹ propranolol infusion was given (n=5) for 10 minutes to reach a steady baseline, before a control hyperoxia was carried out. The adrenaline dose-response was then repeated in the presence of propranolol.

Group 2: Hypercapnic ventilatory sensitivity

The effect of adrenaline on the hypercapnic ventilatory response (CO₂ sensitivity = Δ Vₑ/ mmHg PₐCO₂) was determined (n=5) using a constant infusion of 1µg.kg⁻¹.min⁻¹. The dose was allowed 5 minutes to reach a steady state, before the animal was exposed to graded levels of hypercapnia: 4 and 8% inspired CO₂ for 5 minutes. Adequate time was allowed between
each hypercapnia for recovery to baseline values. Subsequent blood gas analysis revealed that
the presumed 4% level of hypercapnia did not significantly alter $P_aCO_2$ from basal values,
whereas the 8% level gave $P_aCO_2$ levels of $57 \pm 1.3$ mmHg, therefore this level was used
subsequently. This level of hypercapnia was also carried out at baseline, prior to
administration of adrenaline, to obtain a control response. After recovery from adrenaline,
propranolol was given as described above (n=2) and the hypercapnic response assessed. The
adrenaline infusion was then repeated in the presence of propranolol, and the hypercapnia ($61
\pm 4.5$ mmHg) repeated.

### 3.5.2 Group 3: Hyperinsulinaemic hypoglycaemic ramp

Insulin was infused at different rates between 2 - 12 mU.kg$^{-1}$min$^{-1}$ in order to achieve 3 – 4
target levels of hypoglycaemia (blood glucose of 5.7 (basal) to 2.9 mmol/L) (n=6)). Each level
was maintained for around 10 minutes, allowing the effect on basal ventilation to be recorded,
before 30 seconds exposure to hyperoxia was used to determine CB-mediated input. At the
most severe level of hypoglycaemia, the hypercapnic ventilatory response ($CO_2$ sensitivity)
was assessed by exposure to a $P_aCO_2$ of $58 \pm 0.9$ mmHg for 5 minutes. Basal ventilation and
control responses to hyperoxia and hypercapnia were also recorded at euglycaemia. The
protocol was then repeated in the presence of a $0.3mg.kg^{-1}min^{-1}$ propranolol infusion (n=6).
Control responses were carried out after 10 minutes propranolol infusion, before the insulin
infusion was restarted (hypercapnia causing $P_aCO_2$ levels of $56 \pm 1$ mmHg). Blood samples
were taken throughout the ramp protocols with a view to measuring the levels of the counter-
regulatory hormone adrenaline (see section 6 - future work).
3.6 Data analysis

All data is presented as the mean ± SEM of the absolute data or as the difference from baseline or corresponding control response. Graphs were plotted in DeltaGraph 5.7.5, and statistics carried out using GraphPad Prism.

The effect of adrenaline on $V_E$, with hyperoxia and/or propranolol, was plotted as a scatter graph and analysed using repeated measures or one-way ANOVAs as appropriate, with post-hoc Bonferroni tests. Paired or unpaired t-tests were used to compare specific pairs of $V_E$ responses and to analyse the cardiovascular and blood glucose data.

The effect of hypoglycaemia on $V_E$, with hyperoxia and/or propranolol, was plotted as a scatter graph and had linear regression lines fitted. The slope of each data set was analysed statistically using one sample t-tests (to compare each line to zero) and one-way ANOVA with post-hoc Bonferroni was used to compare appropriate pairs of slopes. Paired t-tests were used to analyse the cardiovascular data.

The hypercapnic ventilatory responses under control conditions, in the presence of adrenaline/hypoglycaemia and with or without propranolol, were also plotted as scatter graphs. The slopes of these lines were plotted as bar charts representing CO₂ sensitivity ($\Delta V_E$/mmHg $P_aCO_2$). One-way ANOVAs, with post-hoc Bonferroni tests were carried out to compare the CO₂ sensitivities within each experimental group. Paired and unpaired t-tests were also used to compare specific pairs of data.

The relationship between $P_aCO_2$ and $V_E$ during adrenaline infusion/hypoglycaemia, and the effect of propranolol on this relationship was plotted as a scatter graph. Linear regression lines were fitted to each. The slopes were compared to zero using one-sample t-tests and to each other using unpaired t-tests. All data was analysed to the 95% significance level i.e. $P <0.05$ taken as significant.
4. Results

4.1 Group 1

The effect of adrenaline on ventilation

Figure 9 A shows raw traces of ABP, HR, airflux, $R_t$, $V_t$ and FBF at baseline and during infusion of $1 \mu g.kg^{-1}.min^{-1}$ adrenaline. The main effects seen were increased $R_t$ and $V_t$, as well as increased FBF, MABP and decreased HR. The cardiovascular effects of adrenaline are summarised in Table 1, reflecting the changes shown in the traces, although none reached significance. Adrenaline also caused a significant increase in [blood glucose] from a basal value of $6.6 \pm 0.5$ to $7.3 \pm 0.5 \text{mmol.L}^{-1}$ at $1 \mu g.kg^{-1}.min^{-1}$ (paired t-test, $P < 0.05$).

Figure 9 B shows an adrenaline dose-response curve; adrenaline caused a significant increase in $V_E$ from a basal value of $133.5 \pm 4.1$ to $151.9 \pm 6.8 \text{ml.min}^{-1}$ at the highest dose. However $V_E$ did not increase progressively with each dose tested, only becoming significant at $0.1$ and $1 \mu g.kg^{-1}.min^{-1}$. It was therefore inappropriate to fit a dose-response curve or linear regression line, or to continue repeating the full range of doses, therefore $0$, $0.1$ and $1 \mu g.kg^{-1}.min^{-1}$ were used from this point.

The effect of hyperoxia and propranolol

Figure 10 shows raw traces showing the effects of hyperoxia and propranolol, compared to control. Hyperoxia decreased $R_t$, reflected in the $V_t$ trace. Hyperoxia also significantly decreased HR and FBF. Whilst this trace shows no significant effect on ABP, overall MABP significantly increased, as shown by group mean data in Table 2 (paired t-tests vs control, $P < 0.05$). The major effect of propranolol alone was to cause a significant decrease in HR (cardiovascular data shown in Table 1), as well as a significantly decreased ABP and a slight but non-significant decrease in FVC (as seen in the FBF trace). $R_t$ slightly decreased compared to control, but not by as much as in hyperoxia. There was no significant difference between the HR and MABP with propranolol alone and propranolol with adrenaline infusion.
There was a significant difference in FVC, however the value recorded with propranolol and adrenaline together was not significantly different from the control value (Table 1).

Figure 11 A shows the selected adrenaline doses increasing $V_E$ (i.e. normoxia). Hyperoxia significantly shifted the response down, but $V_E$ still increased with adrenaline. Propranolol also shifted the response down (not as far as hyperoxia), but $V_E$ still increased with adrenaline. However at the top dose, propranolol appears to partly reduce the response, although this did not quite reach significance (unpaired t-test, $P = 0.0519$). Propranolol did not alter the effect of adrenaline on blood glucose as levels still rose, from $6.9 \pm 0.5$ basally, to $7.3 \pm 0.4$ at $1 \mu g.kg^{-1} min^{-1}$ adrenaline, but not significantly. The combination of hyperoxia and propranolol was additive (significances shown on graph from repeated measures ANOVA & post hoc Bonferroni, comparing means of each line).

Figure 11 B shows the effect of hyperoxia, propranolol and the combination, as the difference from the response at each corresponding adrenaline dose. The hyperoxia and combination differences remained fairly stable at each dose, whereas the propranolol difference slightly, but not significantly, increased at the top dose (one-way ANOVAs & post-hoc Bonferroni, $P > 0.05$) reflecting the reduced response in A.

**The effect of adrenaline on $P_aCO_2$**

Figure 12 shows the association between the increased $V_E$ caused by adrenaline (from baseline to $1 \mu g.kg^{-1} min^{-1}$), and the $P_aCO_2$ levels sampled at the corresponding doses. In normoxia i.e. the control response to adrenaline, $V_E$ increased and $P_aCO_2$ remained constant, showing just a slight decrease. In the presence of propranolol, adrenaline still increased $V_E$, but not by as much as in control, as seen in Figure 11 A. However $P_aCO_2$ increased. Statistics could not be carried out on this data as not every animal the ventilation data was drawn from had a corresponding blood gas taken, and vice versa, and so this plot can only suggest a trend from the data available.
4.2 Group 2

The effect of adrenaline on hypercapnic ventilatory sensitivity

Figure 13 shows raw traces demonstrating the response to hypercapnia, the main effect of which was an increased $V_t$, and slightly increased $R_f$. HR and FVC decreased and BP slightly increased in this hypercapnic trace compared to control, but mean group data showed no significant cardiovascular effects of hypercapnia (paired t-tests vs control, $P > 0.05$, shown in Table 3). The 3$^{rd}$ trace shows hypercapnia in the presence of adrenaline; $V_t$ is increased as in the 1$^{st}$ hypercapnic response, but $R_f$ increased further. HR and ABP appear increased compared to the 1$^{st}$ response, which may be accounted for by an effect of the adrenaline.

Figure 14 A shows the hypercapnic ventilatory response at control, and with adrenaline and propranolol. Adrenaline increased basal ventilation and also increased the response to CO$_2$. Propranolol alone and with adrenaline reduced this response to below the control. Figure 14 B shows the slopes of the hypercapnic responses i.e. CO$_2$ sensitivity, demonstrating that there was no significant difference between any of the slopes.

4.3 Group 3

The effect of hypoglycaemia, hyperoxia and propranolol, on ventilation

Figure 15 shows raw traces demonstrating the effect of hypoglycaemia; $R_f$ increased, reflected in the $V_t$ trace. ABP stayed constant, but HR and FBF showed an increase (the 3$^{rd}$ trace shows a hypercapnic response during hypoglycaemia – see Figure 18). However, Table 4 shows that hypoglycaemia had no significant effect on the cardiovascular parameters (paired t-tests vs control, $P > 0.05$). Figure 16 A shows basal ventilation and three levels of insulin-induced hypoglycaemia causing an increased $V_E$. A one-way ANOVA with post-hoc Bonferroni showed no significant increase in $V_E$ overall or between each pair of blood glucose levels.
Hyperoxia shifted the response down, but \( V_E \) still increased at each blood glucose level. Propranolol appeared to prevent the increase, with \( V_E \) actually decreasing. Hyperoxia and propranolol in combination showed an additive effect, shifting \( V_E \) down and preventing the increase. However, as this data was not paired and each animal had slightly different blood glucose values, linear regression fits were deemed more useful in assessing the relationship between hypoglycaemia and \( V_E \). Slopes for each response were determined and plotted as a bar chart in Figure 16 B. Normoxia (the control response to hypoglycaemia) vs. hyperoxia, and propranolol vs. hyperoxia with propranolol were not significantly different, but all other slopes were significantly different from each other (one-way ANOVA and post-hoc Bonferroni, \( P < 0.05 \)) and all slopes were significantly different from 0 (one-sample t tests, \( P < 0.05 \)).

**The effect of hypoglycaemia on \( P_a CO_2 \)**

Figure 17 is a scatter graph showing the association between the increased \( V_E \) caused by hyperinsulinaemic hypoglycaemia, and \( P_a CO_2 \). Under the normoxic conditions i.e. the control response to hypoglycaemia, \( V_E \) increased and \( P_a CO_2 \) remained constant. In the presence of propranolol, \( V_E \) decreased in spite of the hypoglycaemia (as seen in Figure 16 A), and \( P_a CO_2 \) increased. However comparison of the slopes of the fitted linear regression lines showed no significant difference between the two conditions (unpaired t-test, \( P > 0.05 \)), and that neither was significantly different from 0 (one-sample t-tests, \( P > 0.05 \)), potentially due to the large error bars.

**The effect of hypoglycaemia on hypercapnic ventilatory sensitivity**

The 3\(^{\text{rd}}\) raw trace of Figure 15 shows an example hypercapnic response in hypoglycaemia, which resembles the trace seen in hypoglycaemia alone, except \( V_t \) also increased. Figure 18 A shows the control hypercapnic ventilatory response, and the corresponding responses in hypoglycaemia, with propranolol alone and in combination with hypoglycaemia.
Hypoglycaemia increased $V_E$ basally, and caused an increased response to CO$_2$. The response with propranolol was not different from control, but the response during hypoglycaemia and propranolol infusion was blunted to below control levels. The slopes of these response lines provided a measure of CO$_2$ sensitivity, represented by the bar chart in Figure 18 B. The significances shown on the slopes are two-fold: one-way ANOVA with post-hoc Bonferroni showed no significant difference between the control vs hypoglycaemic, control vs propranolol and propranolol vs hypoglycaemia & propranolol CO$_2$ sensitivities, and significant differences between hypoglycaemic/control vs hypoglycaemic & propranolol sensitivities. The animals were not paired between all groups, however a paired t-test could be carried out between control and hypoglycaemia, revealing a significant difference ($P < 0.05$), and an unpaired t-test between hypoglycaemia and hypoglycaemia with propranolol confirmed significance ($P < 0.05$).
Table 1: Cardiovascular effects of adrenaline and propranolol

<table>
<thead>
<tr>
<th>Mean ± SEM</th>
<th>Control</th>
<th>Adrenaline 1µg.kg⁻¹min⁻¹</th>
<th>Propranolol</th>
<th>Adrenaline &amp; propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>449 ± 7.7</td>
<td>448 ± 11.9 (ns)</td>
<td>369 ± 5.4 (*)</td>
<td>374 ± 13.9 (ns)</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>123 ± 2.2</td>
<td>132 ± 4.7 (ns)</td>
<td>109 ± 3.8 (*)</td>
<td>120 ± 7.9 (ns)</td>
</tr>
<tr>
<td>FVC (U)</td>
<td>0.023 ± 0.003</td>
<td>0.026 ± 0.005 (ns)</td>
<td>0.021 ± 0.002 (ns)</td>
<td>0.026 ± 0.002 (*/ns)</td>
</tr>
</tbody>
</table>

Table 1: Mean group cardiovascular data ± SEM. Adrenaline had no significant effect on any of the mean cardiovascular parameters, although MABP and FVC both increased (paired t-tests vs control, P > 0.05). Propranolol alone significantly decreased HR and MABP (P < 0.05) but had no significant effect on FVC (paired t-tests vs control, P > 0.05). Adrenaline had no significant effect on HR or MABP during propranolol infusion, but FVC did significantly increase (paired t-tests propranolol vs adrenaline and propranolol, P < 0.05). However the FVC seen with adrenaline and propranolol was not significantly different to control (paired t-test adrenaline and propranolol vs control, P > 0.05).

Table 2: Cardiovascular effects of hyperoxia

<table>
<thead>
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<th>Mean ± SEM</th>
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<th>Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>449 ± 8.7</td>
<td>438 ± 7.2 (*)</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>123 ± 2.6</td>
<td>129 ± 2.6 (*)</td>
</tr>
<tr>
<td>FVC (U)</td>
<td>0.023 ± 0.003</td>
<td>0.019 ± 0.002 (*)</td>
</tr>
</tbody>
</table>

Table 2: Mean group cardiovascular data ± SEM. Hyperoxia significantly increased MABP, and decreased HR and FVC (paired t-tests vs control, P < 0.05).
Table 3: Cardiovascular effects of hypercapnia

<table>
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<tr>
<th>Mean ± SEM</th>
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<th>Hypercapnia</th>
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</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>449 ± 8.7</td>
<td>425 ± 9.0 (ns)</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>123 ± 2.6</td>
<td>120 ± 3.7 (ns)</td>
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<tr>
<td>FVC (U)</td>
<td>0.023 ± 0.003</td>
<td>0.025 ± 0.003 (ns)</td>
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</tbody>
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Table 3: Mean group cardiovascular data ± SEM. Hypercapnia had no significant effect on HR, MABP or FVC (paired t-tests vs control, P > 0.05).

Table 4: Cardiovascular effects of hypoglycaemia

<table>
<thead>
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<th>Mean ± SEM</th>
<th>Control</th>
<th>Hypoglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>449 ± 8.7</td>
<td>453 ± 10.5 (ns)</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>123 ± 2.6</td>
<td>123 ± 4.5 (ns)</td>
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<tr>
<td>FVC (U)</td>
<td>0.023 ± 0.003</td>
<td>0.024 ± 0.003 (ns)</td>
</tr>
</tbody>
</table>

Table 4: Mean group cardiovascular data ± SEM. Hypoglycaemia had no significant effect on HR, MABP or FVC (paired t-test vs control, P > 0.05).
Figure 9: The effect of adrenaline on ventilation

Figure 9 A: Raw traces showing baseline recordings of ABP, HR, airflux, R, V, and FBF, and the effect of 1µg.kg⁻¹.min⁻¹ adrenaline upon them. The main effects of this adrenaline dose were increased R, V, ABP and FBF, and a decreased HR. Mean cardiovascular data is shown in Table 1. B: An adrenaline dose-response curve; adrenaline significantly increased Vₑ (repeated measures ANOVA, P < 0.05) but not between every dose tested, reaching significance at 0.1 and 1µg.kg⁻¹.min⁻¹ (post-hoc Bonferroni).
Figure 10: The effect of hyperoxia and propranolol (raw traces)

Figure 10: Raw traces showing the effects of hyperoxia and propranolol, compared to control. The main effect of hyperoxia was a decreased $R_f$. Hyperoxia also significantly decreased HR and FBF. Whilst this trace shows no significant effect on ABP, overall MABP significantly increased, as shown by group mean data in Table 1 (paired t-tests vs control, $P < 0.05$). The major effect of propranolol was to cause a significant decrease in HR (cardiovascular data shown in Table 1), as well as a significantly decreased ABP and a slight but non-significant decrease in FVC (as seen in the FBF trace). $R_f$ slightly decreased compared to control, but not as much as with hyperoxia.
Figure 11: The effect of hyperoxia and propranolol

A

The selected adrenaline doses increased $V_E$ (normoxia). Hyperoxia significantly shifted the response down, but $V_E$ still increased with adrenaline. Propranolol also shifted the response down, but not as far as hyperoxia. $V_E$ still increased with adrenaline at the first dose, however at the top dose, propranolol appears to partly reduce the response, although this didn’t reach significance (unpaired t-test, $P = 0.0519$). The combination of hyperoxia and propranolol was additive (significances shown on graph from repeated measures ANOVA & post hoc Bonferroni, comparing means of each line).

B

The effect of hyperoxia, propranolol and the combination, shown as the difference from the response at each corresponding adrenaline dose. The hyperoxia and combination differences remained fairly stable at each dose, whereas the propranolol difference slightly, but not significantly, increased at the top dose (one-way ANOVAs & post-hoc Bonferroni, $P > 0.05$).
Figure 12: The effect of adrenaline on $P_aCO_2$

Figure 12: A scatter graph showing the association between the increased $V_E$ caused by adrenaline (only baseline and top dose shown), and the $P_aCO_2$ levels sampled at the corresponding doses. Under normoxia i.e. the control response to adrenaline, $V_E$ increased and $P_aCO_2$ remained almost constant, showing just a slight decrease. In the presence of propranolol, adrenaline still increased $V_E$, but not by as much as in control, as seen in Figure 11 A. However $P_aCO_2$ increased.
Figure 13: Raw traces showing the response to hypercapnia, the main effect of which was an increased $V_t$, and slightly increased $R_e$. HR and FBF also appear slightly decreased in this example, however none of the mean cardiovascular changes in response to hypercapnia were significant (data shown in Table 3, paired t-tests vs control, $P > 0.05$). The 3rd trace shows hypercapnia in the presence of adrenaline; $V_t$ is increased as in the 1st hypercapnic response, but $R_e$ increased further. HR and ABP appear increased compared to the 1st response, which may be accounted for by an effect of the adrenaline.
Figure 14: The effect of adrenaline on hypercapnic ventilatory sensitivity

A - The effect of hypercapnia on ventilation under different conditions. Adrenaline increased basal ventilation and also increased the response to CO$_2$. Propranolol alone and with adrenaline reduced this response to below the control. B: The slopes of the hypercapnic responses (CO$_2$ sensitivity) shown as a bar chart, demonstrating that the increased response with adrenaline and decreased responses with propranolol (with and without adrenaline) were not significant (one-way ANOVA with post-hoc Bonferroni comparing each group, $P > 0.05$). Propranolol experiments were only carried out in $n = 2$, and so paired and unpaired t-tests were also carried out, also revealing no significance ($P > 0.05$).
Figure 15: The effect of hypoglycaemia, hyperoxia and propranolol, on ventilation (raw traces)

Figure 15: Raw traces showing the effect of hypoglycaemia; $R_t$ increased, reflected in the $V_t$ trace. ABP stayed constant, but HR and FBF showed an increase (3rd raw trace shows a hypercapnic response in hypoglycaemia – see Figure 18). However, as shown in Table 4, hypoglycaemia had no significant effect on the cardiovascular parameters (paired t-tests vs control, P > 0.05).
Figure 16: The effect of hypoglycaemia, hyperoxia and propranolol, on ventilation

Figure 16: A - Linear regression lines showing basal ventilation and three levels of insulin-induced hypoglycaemia causing an increased $V_E$. Hyperoxia shifted the response down, but $V_E$ still increased at each blood glucose level. Propranolol appeared to prevent the increase, with $V_E$ actually decreasing. Hyperoxia and propranolol together showed an additive effect, shifting $V_E$ down and preventing the increase. B: Slopes for each linear regression in A plotted as a bar chart. Normoxia (the control response to hypoglycaemia) vs hyperoxia, and propranolol vs hyperoxia with propranolol were not significantly different, but all other slopes were significantly different from one another (one-way ANOVA and post-hoc Bonferroni, $P < 0.05$) and from 0 (one-sample t-tests, $P < 0.05$).
Figure 17: The effect of hypoglycaemia on $P_a\text{CO}_2$

Figure 17: A scatter graph showing the association between the increased $V_E$ caused by hyperinsulinaemic hypoglycaemia, and $P_a\text{CO}_2$. Under the normoxic conditions i.e. the control response to hypoglycaemia, $V_E$ increased and $P_a\text{CO}_2$ remained constant. In the presence of propranolol, $V_E$ decreased in spite of the hypoglycaemia, and $P_a\text{CO}_2$ increased. However, comparison of the slopes of the fitted linear regression lines showed no significant difference between the two conditions (unpaired t-test, $P > 0.05$), and that neither was significantly different from 0 (one-sample t-tests, $P > 0.05$).
Figure 18: The effect of hypoglycaemia on hypercapnic ventilatory sensitivity

A

- Control
- Propranolol
- Hypoglycaemia
- Hypoglycaemia + propranolol

Figure 18: A - The control hypercapnic ventilatory response, and the corresponding responses in hypoglycaemia, with propranolol alone and in combination with hypoglycaemia. Hypoglycaemia increased $V_e$ basally, and also showed an increased response to $CO_2$. The response with propranolol was not different from control, but the response during hypoglycaemia and propranolol infusion was blunted to below control levels. B: Slopes of each response line in A ($CO_2$ sensitivity), represented as a bar chart. The significances shown on the slopes are two-fold: one-way ANOVA with post-hoc Bonferroni showed no significant difference between the control vs hypoglycaemic, control vs propranolol and propranolol vs hypoglycaemia with propranolol $CO_2$ sensitivities, and significant differences between hypoglycaemic/control vs hypoglycaemic with propranolol sensitivities. A paired t-test between control and hypoglycaemia revealed a significant difference ($P < 0.05$), and an unpaired t-test between hypoglycaemia and hypoglycaemia with propranolol confirmed significance ($P < 0.05$).
5. Discussion

5.1 The effect of adrenaline on ventilation and the hypercapnic ventilatory response

Adrenaline caused a significant increase in $V_E$ when administered as an intravenous infusion at 0.1 and 1µg.kg$^{-1}$min$^{-1}$, supporting a wealth of data regarding the action of adrenaline to increase respiration (e.g. Whelan and Young, 1953, Young, 1957, Joels and White, 1968, Heistad et al, 1972, Maskell et al, 2006). For example, Whelan and Young (1953) showed an increased $R_f$ and $V_t$ after intravenous administration of adrenaline at 10-20µg.min$^{-1}$, which was at its largest during the initial 5 minutes, in line with the infusion length allowed here before respiratory measurements were made. The choice of dose in the present study is justified by the performance of a dose-response curve to observe the effect of a range of doses upon ventilation. The literature reports use of a wide range of doses, although it has been reported that the respiratory response is more readily seen at smaller (µg) doses (Young, 1957), as demonstrated by Linton et al (1992), who showed an increased $V_E$ at 1µg.kg$^{-1}$min$^{-1}$, but no effect at 0.1 µg.kg$^{-1}$min$^{-1}$. No significant effect of adrenaline at the doses used in the present study was seen upon cardiovascular parameters. A slight increase in FVC was seen, which coupled with the lack of effect on blood pressure suggests that the $V_E$ response seen to adrenaline is a direct action on the CB cells rather than an indirect effect via reducing CB blood flow.

Hyperoxia was used to isolate any CB-mediated input to the response to adrenaline, and was seen to reduce the offset in the ventilatory response to adrenaline. However hyperoxia did not block the action of adrenaline (as observed by Joels and White, 1968, and Heistad et al, 1972), as $V_E$ still increased during adrenaline infusion. Propranolol significantly decreased HR, as well as MABP, demonstrating its widely reported role as a β-adrenoreceptor antagonist. The dose used was the same as that used by Maskell et al (2006). The cardiovascular data recorded during concurrent adrenaline and propranolol infusion was inconclusive. HR was still reduced
compared to control and not significantly different to propranolol alone, however MABP increased and FVC increased significantly. This either suggests that the \( \beta \) block was incomplete and so the \( \beta \) action of adrenaline to cause vasodilatation was still able to occur, or that an \( \alpha \) action of adrenaline was responsible. For example adrenaline could have acted via \( \alpha \) receptors to increase MABP (by vasoconstriction), activating a baroreceptor reflex resulting in decreased sympathetic output to muscle. This would cause vasodilatation and therefore an increased FVC. Another possibility is that an \( \alpha \)-mediated metabolic action of adrenaline resulted in release of some by-product which was responsible for the cardiovascular changes. Propranolol showed a similar effect to hyperoxia on \( V_E \), except at 1 \( \mu \text{g.kg}^{-1}\text{min}^{-1} \) adrenaline where the \( V_E \) response appeared slightly (but not significantly) reduced.

Taken together, these data suggest that the action of adrenaline on \( V_E \) is CB independent, or that \( O_2 \) and adrenaline act via independent pathways within CB. This latter possibility is supported by the fact that the response with hyperoxia and propranolol in combination was additive rather than multiplicative, suggesting no interaction between pathways. Previous studies have supported this idea of independent transduction pathways within the CB, for example Zhang et al (2007) showed that the \textit{in vitro} response to hypoglycaemia and hypoxia/hypercapnia were additive, and Garcia-Fernandez et al (2007) showed that a metabolic inhibitor blocked the response to hypoxia but not to low glucose.

It must also be considered whether the hyperoxia applied here was sufficient to ablate CB function, as Kumar et al (2007) reported that a tonic \textit{in vivo} firing could still be seen at \( \text{PO}_2 \)s in excess of 400mmHg, whilst Biscoe et al (1970) reported in vivo single afferent fibre firing up to 600mmHg. Therefore it is likely that the > 300 mmHg \( \text{P}_0\text{O}_2 \) utilised here did not fully ablate CB function, explaining why hyperoxia did not completely prevent the response to adrenaline. This is supported by Heistad et al (1972) who described, in man, that 100\% \( O_2 \)
could block the increased ventilation seen in response to an i.v. infusion of a β-agonist. Additionally they showed that propranolol could also block this ventilatory response, thus further supporting a β- and CB-mediated increase in $V_E$.

Although we were unable to measure metabolic rate in our study, we found that the increased ventilation in response to adrenaline was independent of $P_aCO_2$ thus suggesting that the increased $V_E$ seen was an appropriate hyperpnoea in response to increased metabolic rate, rather than a hyperventilation, as observed by Bin Jaliah et al (2004) and Maskell et al (2006). Whelan and Young (1953) reported a fall in alveolar $CO_2$ and increased $O_2$ consumption during adrenaline infusion, which they described as calorigenic i.e. metabolic, although they also suggested the increased respiration may have been independent of the increased metabolic rate.

It is known that increased metabolism can be induced by adrenaline via both adrenoreceptor groups. $\alpha$ receptors activate downstream pathways inducing liver and adipose tissue glycogenolysis and gluconeogenesis, inhibition of insulin release and stimulation of glucagon release from the pancreas. Stimulation of $\beta$ receptors (via increased cAMP levels) can also lead to increased glucagon release and lipolysis (Rang and Dale, 2006). Hence metabolic effects of adrenaline can be primarily attributed to $\alpha$-action, whilst the respiratory actions are suggested to be via $\beta$ receptors, as demonstrated by Heistad et al (1972) and Maskell et al (2006). Therefore, in the current study, adrenaline should have increased both $V_E$ and metabolism, but the primary effect of propranolol would have been upon $V_E$. However the $\alpha$-actions of adrenaline to increase metabolism would have been unaffected (Rang and Dale, 2006, Yeo and Sawdon, 2007), as supported by the fact that blood glucose still increased in the presence of propranolol (although this was not significant, potentially due to the higher baseline levels seen with propranolol alone). This would explain the increased $P_aCO_2$ seen during $\beta$-block. This rise in $P_aCO_2$ would be expected to stimulate central chemoreceptors, and
this increased central chemoreceptor drive is the most likely explanation for the increased $V_E$ which was still seen during adrenaline infusion despite the presence of propranolol. These data, coupled with the lack of cardiovascular effects of adrenaline i.e. no $\alpha$-mediated increase in ABP and no $\beta$-mediated increase in HR, but just a slight increase in FVC, suggests that different tissues can have different sensitivities to adrenaline, possibly via different adrenoreceptor subtypes.

Adrenaline showed a trend to increase CO$_2$ sensitivity in the present experiment, increasing the hypercapnic ventilatory response, which could subsequently be reduced by propranolol. Maskell et al (2006) have also shown that adrenaline (at a higher dose of 10$\mu$g.kg$^{-1}$min$^{-1}$) increases CO$_2$ sensitivity, and that this was abolished by CSNX, hence it was CB-mediated. They also showed the increased chemosensitivity was blocked by infusion of propranolol (0.3mg.kg$^{-1}$min$^{-1}$, as used here), supporting the present data, and showing that the effect was $\beta$-mediated. Along with the present data showing no change in $P_a$CO$_2$ with the increased $V_E$ associated with adrenaline infusion, and that this hyperpnoea could be prevented by propranolol, the trend for an increased CO$_2$ sensitivity supports the hypothesis by Maskell et al (2006); that adrenaline may be one of the blood-borne factors that mediate hyperpnoea by an action on CO$_2$ chemosensitivity.

5.2 The effect of hyperinsulinaemic hypoglycaemia on ventilation

A decreased blood glucose caused an increased $V_E$, with a linear regression slope significantly different to zero. This has been shown previously by Bin Jaliah et al (2004, 2005) and Ward et al (2007). Bin Jaliah et al (2004) isolated the role of the CB, showing reduced basal $V_E$ and no increased $V_E$ in response to hypoglycaemia in CSNX animals, supporting a role for the CB in mediating the increased $V_E$ seen.
It has previously been shown that hypoglycaemia is associated with an increased release of counter-regulatory hormones including adrenaline (Vollmer et al., 1997, Koyama et al., 2000, 2001, Ward et al., 2007, Yeo and Sawdon, 2007, Wehrwein et al., 2010). Both the increased $V_E$ and counter-regulatory hormone release has been shown to be reduced by CBR (Koyama et al., 2000, 2001) or application of hyperoxia (Wehrwein et al., 2010), suggesting a CB-dependency of the effect. This mechanism explains the need for an increased glucose infusion to clamp plasma glucose during hyperinsulinaemia, in absence of CB input (Koyama et al., 2000, 2001, Wehrwein et al., 2010). These findings are consistent with the results of the present study, where hyperoxia reduced the ventilatory response to hypoglycaemia. However, hyperoxia did not block the response, with a constant difference between normoxic and hyperoxic $V_E$ at each blood glucose resulting in a slope that was offset, but not different from control (normoxia). However propranolol was able to abolish the increase in $V_E$ that was seen in hypoglycaemia. This suggests that the rise in $V_E$ associated with hypoglycaemia is $\beta$-dependent. Taken in combination with the hyperoxia data, this suggests that the increased $V_E$ in hypoglycaemia is either a CB independent mechanism, or that an $O_2$-independent pathway exists within the CB for sensing hypoglycaemia (supported by Garcia-Fernandez et al., 2007 and Zhang et al., 2007, as discussed above). The fact that the effect of hyperoxia and propranolol together appeared additive (slope not significantly different from propranolol alone), supports this idea of independent pathways within the CB.

As mentioned above, given the reports that in vivo CB afferent firing can still be seen at $P_{aO_2}$s of up to 400-600mmHg (Kumar, 2007, Biscoe et al. 1970), it is likely that the level of hyperoxia used here did not fully ablate CB function, offering further explanation as to why a $V_E$ response to hypoglycaemia was still seen and supporting a CB-dependent mechanism.
This is further supported by the evidence for a CB role in glucosensing (see section 1.4), and it therefore seems unlikely that these data represent a CB-independent ventilatory response to hypoglycaemia.

The data therefore disagrees with the results of \textit{in vitro} studies that suggest the CB responds to low glucose directly (Pardal and Lopez-Barneo, 2002, Lopez-Barneo, 2003, Garcia-Fernandez \textit{et al}, 2007, Zhang \textit{et al}, 2007). In addition the present data contradicts the speculations from \textit{in vivo} studies such as Koyama \textit{et al} (2000, 2001) and Wehrwein \textit{et al} (2010), which suggest, but provide no direct evidence for, low glucose acting directly on the CB. It also offers \textit{in vivo} support for those \textit{in vitro} studies that showed no effect of low glucose on chemoafferent discharge; despite showing an \textit{in vivo} increase in $V_E$ during hypoglycaemia, which was ablated by CSNX (Bin Jaliah \textit{et al}, 2004, 2005). Based on this data, Bin Jaliah \textit{et al} (2004, 2005) suggested a potential role for one of the associated counter-regulatory hormones as a blood-borne mediator sensed by the CB, a possibility that was also recognised by Ward \textit{et al} (2007). Given the present data showing that propranolol was able to block the $V_E$ response to hypoglycaemia, this blood-borne mediator may be adrenaline.

The relationship seen between hypoglycaemia and $P_aCO_2$ suggests that the increased $V_E$ seen was a hyperpnoea rather than a hyperventilation, as $P_aCO_2$ remained constant. This suggests that the hyperinsulinaemic hypoglycaemia caused an increased metabolism, which is consistent with previous findings by Bin Jaliah \textit{et al} (2004, 2005). In the present study, $P_aCO_2$ fell slightly, as was also seen in response to adrenaline. However, this fall does not appear large enough to be explained by a hyperventilation. For example, a fall in $P_aCO_2$ of around 5mmHg was observed in response to a 20% increase in $V_E$ generated in response to graded hypoxia (i.e. a hyperventilation) (Marshall and Metcalfe, 1988), whereas we only observed a fall of around 1mmHg in response to a hypoglycaemia- (and presumably adrenaline-)

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mediated 15% increase in ventilation. This is in line with data reported by Forster and Pan (1994), where $P_aCO_2$ only changes by 1-2mmHg in human exercise (a situation of increased metabolism).

In the presence of propranolol, both hypoglycaemia and adrenaline did not induce as much of an increased $V_E$, and $P_aCO_2$ increased. This suggests that propranolol prevented the increase in $V_E$ that is usually matched to the increased metabolism i.e. it occurs via a β-mediated mechanism, whilst metabolism was still able to increase via the action of adrenaline at α receptors, as well as via the other associated counter-regulatory hormones in hypoglycaemia. However, the data presented did not show statistically significant results, possibly due to the high variability associated with the $V_E$ data.

**The effect of hyperinsulinaemic hypoglycaemia on hypercapnic ventilatory sensitivity**

Bin Jaliah et al (2005) showed an increased CO$_2$ chemosensitivity during hypoglycaemia which was reduced by ~75% after CSNX, presenting a possible CB-mediated mechanism by which $P_aCO_2$ could remain constant during increased metabolism. Increasing the sensitivity of the chemoreceptors for CO$_2$ would lead to an increased $V_E$, facilitating matching to the increased CO$_2$ production associated with increased metabolism. This was demonstrated in the present study, where the ventilatory response to hypercapnia was increased during hypoglycaemia, showing a significantly increased CO$_2$ sensitivity. Bin Jaliah et al (2005) also showed that a fall in glucose concentration decreased CO$_2$ sensitivity in an *in vitro* CB preparation, suggesting that the *in vivo* increase in CO$_2$ sensitivity and hyperpnoea was not via a direct action of low glucose. This was supported by the present finding that propranolol was able to block, and even significantly reduce, the increased CO$_2$ sensitivity associated with hypoglycaemia, suggesting a β-mediated mechanism i.e. adrenaline.
5.3 Comparison of the responses to adrenaline and hypoglycaemia

The response to application of exogenous adrenaline and hyperinsulinaemic hypoglycaemia are similar; both causing an increase in $V_E$ of similar magnitude (~20ml.min$^{-1}$). Both responses were affected similarly by application of hyperoxia, i.e. $V_E$ was reduced, but still showed an increase with increasing stimulus intensity. This may be explained by the fact that the CB was not completely switched off by the hyperoxia, or, based on the assumption that CB activity was reduced, suggests that the responses were either independent of the CB or sensed via $O_2$ independent pathways within the CB. Propranolol had differing effects, causing a small but non-significant decrease in the $V_E$ response to $1\mu g.kg^{-1}min^{-1}$ adrenaline, but causing a complete attenuation of the increased $V_E$ caused by hypoglycaemia, with $V_E$ even decreasing. The application of hyperoxia and propranolol in combination had an additive effect in both experiments, supporting the idea of independent pathways within the CB for different stimuli i.e. $O_2$ and adrenaline/low glucose.

The maintenance of $P_aCO_2$ during the increased $V_E$ caused by both adrenaline and hypoglycaemia, suggested that metabolic rate had increased and the observed increase in $V_E$ was a hyperpnoea as opposed to a hyperventilation. In the presence of propranolol in both experimental groups, $P_aCO_2$ showed a tendency to rise, although this did not reach statistical significance in either group. These data suggest that metabolism rises via an $\alpha$-receptor mechanism, whilst matching $V_E$ to this metabolism is $\beta$-dependent.

Finally, $CO_2$ sensitivity was increased during infusion of adrenaline and significantly during insulin-induced hypoglycaemia, which would provide a mechanism for the ability to maintain isocapnia in the face of increased metabolism.
Based on these findings and the knowledge that adrenaline is a major counter-regulatory hormone released in response to hypoglycaemia (Vollmer et al, 1997, Koyama et al, 2000, 2001, Yeo and Sawdon, 2007, Ward et al, 2007, Wehrwein et al, 2010), it would not be unreasonable to conclude that the hyperinsulinaemic hypoglycaemia had caused endogenous release of adrenaline, and that this adrenaline caused the respiratory responses observed via β-receptors, as the response was reduced by propranolol. When taken alongside previous studies already discussed, which have suggested the CB does not respond to low glucose directly (e.g. Almaraz et al, 1984, Conde et al, 2007, Fitzgerald et al, 2009) but to some other blood borne mediator (Bin Jaliah et al, 2004, 2005) and that adrenaline can increase VE and CO₂ sensitivity via CB-dependent mechanisms (Heistad et al, 1972, Maskell et al, 2006), it may be suggested that this data demonstrates a β-receptor and CB-dependent role for adrenaline in situations of increased metabolism.

A physiological example of such a situation is exercise. The CBs have been shown to play a neuroendocrine role during exercise, as demonstrated by Koyama et al (2001). They showed that CBR dogs had reduced levels of glucagon and noradrenaline released during exercise compared to sham animals, and a mismatch between glucose use and production, thus also linking back to the proposed role in glucoregulation.

An immediate matching of VE to metabolism is seen in exercise, maintaining isocapnia or slight hypocapnia, as well as normoxia and normal arterial pH. Although peripheral chemoreceptors are not thought to be the major mediators of the hypercapnic response, they do give a rapid response to increased PCO₂, increasing VE. The timing of this response therefore fits with the almost immediate increase in VE seen during exercise. Based on the present experiment and literature, this fast response to increased CO₂ from increased metabolism may be mediated by an increased CB CO₂ chemosensitivity, which is potentially mediated by adrenaline, either directly or indirectly via increased sympathetic activity.
Further implicating the CB in exercise hyperpnoea is the evidence that the response is depressed in CBR subjects compared those with intact carotid bodies (Honda, 1985, Wasserman et al, 1975).

The ventilatory response to exercise is in three phases: phase I shows the rapid increase in $V_E$ that occurs at the start of the exercise, or even in anticipation before exercise begins, phase II; an exponential increase in $V_E$ during dynamic exercise, and phase III; a steady-state of $V_E$.

It is recognised that the major cardiovascular and respiratory controller in exercise is a central feed-forward mechanism. Afferent reflexes then provide additional input e.g. respiratory muscle metaboreceptors, locomotor muscle afferents and the CBs (for review see Dempsey, 2012). It has been proposed that the CBs are involved in phases II and III, maintaining $P_{aCO_2}$ and preventing hypoxia, providing around a 20% proportion of the ventilatory drive in phase III (Ward, 1994, Whipp, 1994). However, Dempsey and Smith (1994) have shown in various animal models that the CB inhibits respiratory motor output during heavy exercise, and Forster and Pan (1994) ascribed the CB a role in ‘fine tuning’ ventilation during submaximal exercise, but no major role in exercise hyperpnoea.

5.4 A possible role for potassium

It has long been shown that adrenaline can cause a decrease in plasma K$^+$ levels (D’Silva, 1934), although some have reported an increase in K$^+$ associated with adrenaline infusion (Linton et al, 1992). It has been observed that this plasma K$^+$ lowering action of adrenaline is via a $\beta_2$-receptor dependent action on the Na$^+$/K$^+$ ATPase pump, moving K$^+$ into cells (Clausen, 1983). This is particularly important in exercise, when K$^+$ leaks from cells and the adrenaline released in association with exercise acts to counter this hyperkalaemia (Lancet editorial, 1983, Clausen, 1983). Insulin can have the same effect as adrenaline on K$^+$, as observed by Bin Jaliah et al (2004), who reported a decrease in arterial K$^+$ levels during a
hyperinsulinaemic clamp protocol. However this is via a non-β dependent mechanism (Minaker and Rowe, 1982).

In relation to the current experiment, adrenaline infusion would have driven K\(^+\) into cells, causing hyperpolarisation of all cells, including CB type I cells. This would increase the threshold for excitation of the CB and may explain why the dose-response of adrenaline against \(V_E\) was complex. In the presence of propranolol, any direct β effect of adrenaline on the CB to increase \(V_E\) would be lost. Additionally, the action of adrenaline on the \(\text{Na}^+/\text{K}^+\) ATPase acting via \(\beta_2\) receptors would also be blocked, thus unmasking any effect of K\(^+\). This is supported by the observation that β-blockers can cause hyperkalaemia, especially during exercise when adrenaline levels are increased (Clausen, 1983). Band and Linton (1986) showed that an infusion of KCl produced hyperkalaemia in cats similar to that seen during exercise, resulting in a significantly increased carotid body chemoafferent firing. The K\(^+\) levels increased the level and amplitude of oscillations in CB afferent firing which aligned with respiration and have previously been suggested to play a role in the control of breathing during exercise (Band et al, 1978, 1980, Kumar et al, 1988). It was therefore suggested that K\(^+\) may underlie this role (Band and Linton, 1986, Nye, 1994). This may explain why adrenaline still caused an increase in \(V_E\) during propranolol infusion.

Insulin also drives K\(^+\) plasma levels down in a dose-dependent manner, via the \(\text{Na}^+/\text{K}^+\) ATPase, but via a non-β mediated mechanism (Minaker and Rowe, 1982). This means that the hyperinsulinamic hypoglycaemia would have also been accompanied by a hypokalaemia, which would then be further accentuated by the release of counter-regulatory adrenaline. Hence the increased \(V_E\) seen was a balance between the effect of increased adrenaline and low K\(^+\). When propranolol was given in hypoglycaemia, the effect of adrenaline on K\(^+\) would be removed; however the β-independent effect of insulin would still be present, meaning that
plasma K$^+$ levels would continue to decrease. In this situation, propranolol is presumably blocking the adrenaline drive to $V_E$, and insulin is reducing the K$^+$ drive (unlike the situation with adrenaline and propranolol). This may therefore offer an explanation as to why $V_E$ was seen to decrease with propranolol during hypoglycaemia.

5.5 Methodological limitations

Limitations are recognised within the experiments carried out. For instance a control hyperinsulinaemic euglycaemic clamp was not carried out to control for effect of the high insulin infusion itself. However this has been carried out previously by co-workers (Bin Jaliah et al, 2004, 2005) who showed no direct effect of insulin on the CB.

A steady $P_a$CO$_2$ during an increased $V_E$ was taken to indicate a hyperpnoea in the presence of increased metabolism. However, use of plethysmography or a spirometer attached to a sealed system would have allowed for evaluation of O$_2$ consumption or CO$_2$ production, which are indicators of metabolic activity.

Total $V_E$ was used to here to represent the respiratory response to adrenaline, hypoglycaemia and hypercapnia; however there was no determination of physiological dead space. The amount of gas that was available for gas exchange, the alveolar $V_E$ ($V_A$) would have been smaller than the data reported. We did not record this and therefore have had to assume $V_A$ to be a constant fraction of total $V_E$.

Hyperoxia was used in this investigation to ‘chemically denervate’ the CB. However it has been shown that even at a $P_a$O$_2$ of 400-600mmHg, some in vivo basal firing remains (Kumar, 2007, Biscoe et al, 1970) and if CO$_2$ is not controlled for, there will still be CB activity (see section 1.2 - hypercapnia). Therefore it must be accepted that the hyperoxia used here
probably reduced but did not ablate CB activity. This may explain why hyperoxia did not prevent the $V_E$ increases seen in response to adrenaline and hypoglycaemia, supporting a role for the CB in mediating the observed responses.
6. Future work

Based on the findings of the current study, several pieces of future work could be carried out to help consolidate the conclusions and address the ideas raised by the data:

Measurement of plasma adrenaline from the blood samples taken during the hyperinsulinaemic hypoglycaemic ramp should be carried out in order to confirm the release of adrenaline and compare the concentration seen to the dose given exogenously in the first set of experiments. Koyama et al (2000) reported adrenaline concentrations of around 300pg.ml\(^{-1}\) in response to hypoglycaemia, much lower than the exogenous dose of 1µg.kg\(^{-1}\)min\(^{-1}\) administered here. It may also be prudent to repeat the adrenaline dose-response protocol, taking blood samples at each dose to measure the concentration of adrenaline in the blood, e.g. using HPLC as performed by Maskell et al (2006). The exogenous dose given would have been subject to possible uptake mechanisms and metabolism, and so does not represent the circulating levels.

The experiments which used hyperoxia to isolate the role of the CB could be repeated using CSNX or CBR, surgically removing CB input and allowing comparison with the current results. This would determine whether the level of hyperoxia used was ablating all of the CB input and therefore if the increased \(V_E\) and \(CO_2\) sensitivity seen during exogenous adrenaline infusion and hyperinsulinaemic hypoglycaemia are CB-dependent mechanisms.

The experiments could also be repeated using a \(\beta\)-agonist such as isoprenaline, in order to reconcile the inconclusive cardiovascular data seen here with adrenaline and propranolol.

Considering the potential role of \(K^+\) in the increased \(V_E\) seen with adrenaline and hypercapnia, an improvement for future experiments would be to have the ability to measure \(K^+\) levels. This can often be determined from the sample taken for blood gas analysis, and so would not require further blood sampling or an increased protocol time.
As it is known that the CB possesses functional plasticity, changes in chemoreceptor sensitivity may occur in response to conditions with exposure to chronic/intermittent hypoxia such as obstructive sleep apnoea. These diseases may be associated with the co-morbidity of diabetes, presenting an opportunity for the possible alteration of the glucoregulatory response. Investigation of such conditions in terms of CB function may offer new therapeutic targets.
7. Conclusion

This study provides evidence for a role of adrenaline, a counter-regulatory hormone released in response to hypoglycaemia, in the hyperpnoea of hypermetabolism. The data generated thus supports a body of literature that suggests that the ventilatory response to hypoglycaemia is not mediated by a direct action of low glucose on the CB, but the action of an associated blood-borne mediator.

The observation that CO$_2$ sensitivity was augmented during adrenaline infusion and hypoglycaemia, offers a mechanism by which V$_E$ could be matched to metabolism in the absence of a change in P$_a$CO$_2$. Reduction of the increased V$_E$, CO$_2$ sensitivity and hyperpnoea by the β-antagonist propranolol confirms the β-dependence of the mechanism. Further experiments are required to confirm whether adrenaline acts via the CB, in an O$_2$ independent manner or whether the level of hyperoxia used was able to fully isolate the role of the CB.
8. References


