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Research article

Hypoxia signalling and regulation in chemosensory behaviour of *Caenorhabditis elegans*

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Adaptation to hypoxia is essential to survival in most organisms; disruption of oxygen homeostasis is linked to the pathology of multiple diseases including neurodegeneration, ischaemic stroke and cancer. Hypoxia-inducible factor 1 (HIF-1) is a key transcription factor in the detection of oxygen depletion and in mediating the response to hypoxia to maintain cellular oxygen homeostasis. This study investigated hypoxia signalling *in vivo* using a *Caenorhabditis elegans* HIF-1 mutant model. The chemosensory behaviour of *C. elegans* was analysed through the use of chemosensory assays with a chemoattractant and a chemorepellent; the response was quantified by calculating the chemotaxis index of species. Chemosensory assays were used to analyse behavioural changes of *C. elegans* under oxidic and hypoxic conditions and to analyse the effects of mood stabilizing drugs lithium chloride (LiCl) and valproic acid (VA). HIF-1 mutant *C. elegans* showed an impaired chemosensory response to a 48 h hypoxia exposure. Treatment with LiCl significantly rescued the chemosensory response of HIF-1 mutants under hypoxia, suggesting a protective effect. Treatment with VA decreased the chemosensory response of HIF-1 mutants with hypoxia exposure. Interestingly, VA also decreased the chemosensory response of wild-type species under oxidic conditions, suggesting a mechanism of action independent of hypoxia.

Key words: hypoxia, *C. elegans*, HIF-1, gsk-3 β , lithium chloride, valproic acid

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Introduction

Oxygen regulation is essential for maintaining normal cellular function and for a diverse range of biological processes including embryonic development and physiological homeostasis. Low oxygen levels, or hypoxia, in cells and tissues are linked to the pathology of many diseases including neurodegeneration, ischaemic stroke and tumour growth and metastasis. The ability to adapt to hypoxic conditions is essential for survival in almost all organisms. The transcription factor hypoxia-inducible factor 1 (HIF-1) is a key regulator in the detection and response to cellular hypoxia. HIF-1 induces the transcription of genes that are essential to oxygen transport, glucose metabolism, cell proliferation, cell survival and angiogenesis (Majmudar *et al.*, 2010).

HIF-1 plays a central role in sensing hypoxic environments and regulating gene expression to maintain cellular oxygen homeostasis. The HIF-1 heterodimer comprises an alpha (HIF-1 α , 120 kDa) and a beta (HIF-1 β 91–94 kDa) subunit. The HIF-1 β subunit is constitutively expressed in cells in contrast to the HIF-1 α subunit whose stability is sensitive to oxygen deprivation. HIF-1 α is degraded under normal cellular oxygen levels but is stabilized and active under hypoxic conditions. HIF-1 α stability under oxidic conditions is negatively regulated by prolyl hydroxylase domain (PHD) proteins that induce hydroxylation within highly conserved oxygen-dependent degradation (ODD) domains at specific proline residues (Pro402 and Pro564) of HIF-1 α . This hydroxylation is required for binding of the von Hippel-Lindau

protein (pVHL) ubiquitin E3 ligase complex that targets HIF-1 α for ubiquitination and proteasomal degradation (Ivan *et al.*, 2001; Mahon, Hirota and Semenza, 2001; Yu *et al.*, 2001; Chan *et al.*, 2005). Under hypoxic stress, the oxygen-sensitive HIF-1 α subunit is stabilized in the nucleus by the association of the HIF-1 β subunit and the co-activators CREB-binding protein (CBP) and p300. The factor inhibiting HIF-1 (FIH-1) protein induces hydroxylation of asparagine residue 802 (Asn802) on HIF-1 α which blocks binding of the co-activators CBP and p300 under oxic conditions, targeting it for proteasomal degradation. Under hypoxia, the newly formed and stable HIF-1 α /HIF-1 β /CRB/p300 complex binds directly to the consensus DNA sequence 5'-(A/G)CGTG-3' within the *cis*-acting hypoxia response element (HRE) of target genes resulting in gene transcription (Semenza, 2014). The expression of over 100 target genes has been identified to be transactivated by HIF-1 α in response to hypoxia (Liu *et al.*, 2012). Proteins encoded by HIF-1 target genes allow adaptation to hypoxia through balancing increased oxygen delivery to cells and decreased oxygen consumption of cells (Semenza, 2014).

Glycogen synthase kinase-3 β is a transcriptional regulator of HIF-1 α

GSK-3 is a constitutively active ubiquitous serine-threonine kinase, consisting of α and β isoforms; it is involved in many signalling pathways including the PI3K/Akt pathway. The PI3K/Akt signalling pathway and phosphorylation of GSK-3 β have been reported to regulate the expression and stabilization of HIF-1 α during hypoxia. Downstream signalling induces PI3K-mediated activation and phosphorylation of Akt at Ser473. Phosphorylated Akt (p-Akt) directly phosphorylates downstream targets including GSK-3 β , rendering it inactive. GSK-3 β acts as a transcriptional regulator of HIF-1 α expression; the active conformation of GSK-3 β directly phosphorylates HIF-1 α and targets it for proteasomal degradation and the inactive form stabilizes HIF-1 α . Hypoxia has been shown to both activate (Beitner-Johnson *et al.*, 2001) and inhibit (Loberg, Vesely and Brosius, 2002) PI3K/Akt-induced phosphorylation of GSK-3 β . Consequently, the interaction between GSK-3 β activity and hypoxia can influence multiple cellular processes including gene expression, protein synthesis, cell proliferation, cell survival and apoptosis (De Sarno, Li and Jope, 2002). Hypoxia-induced p-Akt phosphorylation of GSK-3 β can result in increased HIF-1 α stability, which has been implicated as a survival mechanism of cancer cells by encouraging angiogenesis and metastasis in the hypoxic tumour microenvironment (Chen *et al.*, 2001). Alternatively, studies have also shown that hypoxia can increase the level of active GSK-3 β after ischaemic stroke injury, resulting in an inability to adapt to hypoxia and increased apoptotic activity and cell death.

Lithium chloride is an inhibitor of GSK-3 β

Interest surrounding drugs that can alter the cellular response to hypoxia through altering the stability and expression of

HIF-1 α is increasing. The mood stabilizing drugs lithium chloride (LiCl) and valproic acid (VA) have been implicated as potential novel therapeutics for cancer, neurodegenerative diseases and ischaemic injury due to their effects on HIF-1 α stability and expression. LiCl is currently used to treat bipolar disorders and has been shown to increase the stabilization of HIF-1 α through indirect and direct inhibition of GSK-3 β resulting in an increased ability of cells to adapt to hypoxia (Fig. 1) (Gould and Manji, 2005). LiCl competes for the magnesium-binding site on GSK-3 β , directly inhibiting its ATP-magnesium-dependent catalytic activity. LiCl indirectly inactivates GSK-3 β by increasing PI3K/Akt-mediated Ser9 phosphorylation of GSK-3 β and by disrupting the formation of the β Arr2/PP2A/Akt complex that functions as a negative feedback loop to dephosphorylate Akt (Beurel, Michalek and Jope, 2010). The use of LiCl to inhibit GSK-3 β is thought to prevent phosphorylation and targeted degradation of HIF-1 α resulting in increased stability of HIF-1 α which may aid in the adaptation to hypoxia after ischaemic injury, PI3K/Akt signalling dysfunction or GSK-3 β overexpression (Chuang, Wang and Chiu, 2011).

The mood stabilizing drug VA has also been shown to interfere with HIF-1 α stabilization by inhibiting its accumulation (Machado-Vieira, Ibrahim and Zarate, 2011). VA is an inhibitor of histone deacetylase (HDAC); histones are deacetylated at lysine residues by HDACs resulting in increased affinity of binding between histones and the DNA backbone (Kim *et al.*, 2007). Inhibition of HDACs causes hypoacetylation and subsequent gene silencing. VA acts to inhibit the actions of HDAC resulting in a down-regulated expression of HIF-1 α and an inability to adapt to hypoxic conditions.

The complex interactions between GSK-3 β activity, HIF-1 α and hypoxia are not fully understood. The mechanisms of action of LiCl and VA on GSK-3 β activity and HIF-1 α stability with hypoxia also remain unclear. LiCl in particular is a multi-target inhibitor, of which many interactions with signalling pathways remain unknown. One of the objectives of this study was to explore these connections further using *C. elegans* for *in vivo* analysis of chemosensory behaviour with exposure to hypoxia and treatment with LiCl and VA.

Using *C. elegans* as a model

Caenorhabditis elegans is a useful model in understanding evolutionarily conserved signalling pathways including those involved in the HIF-1-mediated response to hypoxia. The *hif-1* gene encodes HIF-1 in *C. elegans* which allows adaptation to oxygen deprivation through targeted gene expression. HIF-1 stability in *C. elegans* is negatively regulated by the conserved EGL-9/VHL-1 pathway by ODD. The *C. elegans* *egl-9* gene and *vhl-1* gene, orthologous to mammalian PHD genes and pVHL genes, respectively, are involved in the proteasomal degradation of HIF-1 (Metzen *et al.*, 2005).

Caenorhabditis elegans is an important genetic and neuropharmacological model in understanding hypoxic response as it allows for direct correlations between genotype and

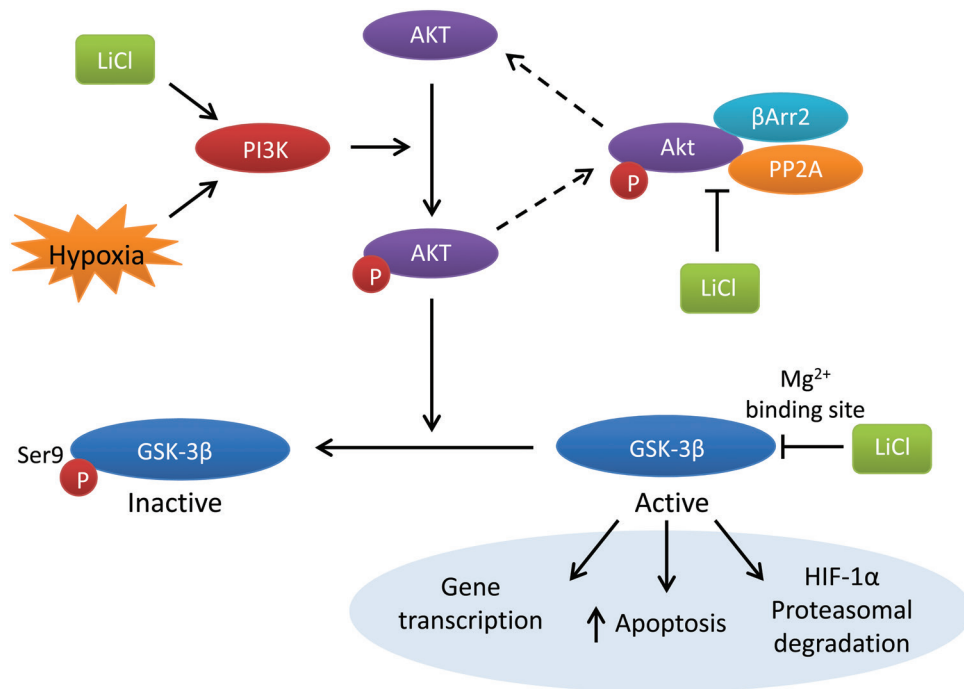


Figure 1. Direct and indirect inhibition of GSK-3 β by lithium chloride (LiCl). LiCl competes for the magnesium binding site on GSK-3 β causing direct inactivation. LiCl increases GSK-3 β phosphorylation at serine 9 (Ser9) through increased PI3K/Akt activation, resulting in inhibition of GSK-3 β . LiCl also reduces inactivation of p-Akt by disrupting the formation of the β Arr2/PP2A/Akt complex, a negative feedback loop for dephosphorylation of Akt, the dashed line represents reduced pathway activity as a result of LiCl. Hypoxia can activate PI3K phosphorylation of Akt resulting in the inactivation of GSK-3 β and an increased stability of HIF-1 α . Adapted from Chiu and Chuang (2010), with permission from Elsevier.

phenotype to be made. Chemosensory assays allow analysis and quantification of behavioural changes in *C. elegans* based on their use of chemosensation to find food and avoid noxious stimuli to develop and reproduce. The chemosensory neurons of *C. elegans* allow exposure of their sensory cilia to the environment which can be utilized in chemosensory assays by using a chemoattractant and a chemorepellent to observe behavioural changes (Bargmann, Hartwig and Horvitz, 1993). Whereby WT strains are expected to avoid the chemorepellent and move towards the chemoattractant.

Materials and methods

Materials

All materials and reagents were purchased from Fisher Scientific unless stated otherwise.

Caenorhabditis elegans strains

Two strains of *C. elegans* were used; N2 (ancestral) wildtype (WT) *C. elegans* strain and a HIF-1(ia4) mutant strain. Both strains were provided by the University of Bath. Frozen strains were thawed from liquid nitrogen storage and grown on Nematode Growth Medium (NGM) agar plates seeded with *E. coli* OP50.

Maintenance of *C. elegans*.

Both WT and HIF-1 mutant strains were maintained under the same stable temperature and light conditions. Cultures were kept in incubation at 18°C with *ad libitum* food source. Nematodes were grown on 90 mm NGM agar plates seeded with 200 μ l *Escherichia coli* OP50 in LB broth as a food source. Nematodes were transferred to fresh NGM OP50 plates using the 'chunking' method with a sterile loop and aseptic techniques.

Passaging of *C. elegans* cultures was carried out intermittently (approximately once a week) to avoid undesirable conditions such as overcrowding and food depletion, to limit the formation of dauer worms and to optimize the number of nematodes available for chemosensory assays. Dauer worms can survive in undesirable conditions and would therefore influence the results of assays, so formation of these was avoided.

Synchronization of *C. elegans*

Synchronization of *C. elegans* cultures was carried out to ensure that nematodes were in the same developmental stage (L3-L4) for exposure to hypoxia or oxia and for the chemosensory assays. Synchronization was achieved using a bleaching protocol. *Caenorhabditis elegans* were left for 38–40 h after bleaching before exposure to hypoxic or oxic conditions.

Hypoxia exposure

The hypoxic environment was achieved through the use of a hypoxia chamber with a 1% O₂/5% CO₂ gas mixture at a flow rate of 5 cm³/min; 1% oxygen levels were monitored with an oxygen sensor, ±0.1% sensitivity (Vandagraph, UK). *Caenorhabditis elegans* cultures on NGM agar plates seeded with *E. coli* OP50 were exposed to either hypoxic or oxic conditions for 48 h prior to chemosensory assays. Both exposure conditions were kept under the same stable temperature (25°C) and light conditions.

Lithium chloride and valproic acid treatment

Nematodes were exposed to 3 mM VA and 3 mM LiCl for 48 h prior to chemosensory assays in parallel with 48 h exposure to hypoxia or oxia (McCull et al., 2008). LiCl and VA were dissolved in NGM after autoclaving and poured into 50 mm plates. Plates were then seeded with 100 µl OP50 and stored at 4°C overnight. Synchronized cultures of *C. elegans* were then transferred onto these treatment plates for 48 h drug exposure.

Chemosensory assays

Chemosensory assays were used to analyse and quantify the phenotypic behaviour of *C. elegans* WT and HIF-1 mutant strains after hypoxic or oxic exposure and to identify changes

in chemosensory behaviour of *C. elegans* upon treatment with LiCl and VA.

Fifty millimetre NGM agar plates, without OP50, were divided into four quadrants (A–D) by marking the bottom of the plates (Fig. 2A). *Caenorhabditis elegans* were transferred from culture plates via the M9 buffer washing protocol after exposure to hypoxia or oxia and treatment with LiCl or VA. Approximately 20 µl of worm suspension was transferred per plate to the assay plates, depending on the number of worms recovered from the plates after exposure conditions (between 20 and 40 µl). Twenty per cent butanone was used as a chemoattractant (Kauffman et al., 2011) and 0.1% sodium dodecyl sulphate (SDS) was used as a chemorepellent (Hilliard et al., 2002); M9 buffer was used as a control. A 1:10 dilution of sodium azide was added to butanone and SDS to immobilize *C. elegans*, so they remained in the chosen quadrant to allow for subsequent counting and calculation of chemotaxis index (CI) (Margie, Palmer and Chin-Sang, 2013). Temperature and light were kept at a constant throughout the assays.

Worms were counted in each quadrant 60 min after commencing the assays. Preliminary tests using 20, 40 and 60 min time points showed that 60 min allowed sufficient time for movement of worms to have occurred. Counts at 20 and 40 min did not allow enough time for worms to move from the centre starting point; therefore, 60 min was used as a time point for subsequent assays. CI was calculated for each assay and used to quantify the chemotactic response of the worms (Fig. 2B).

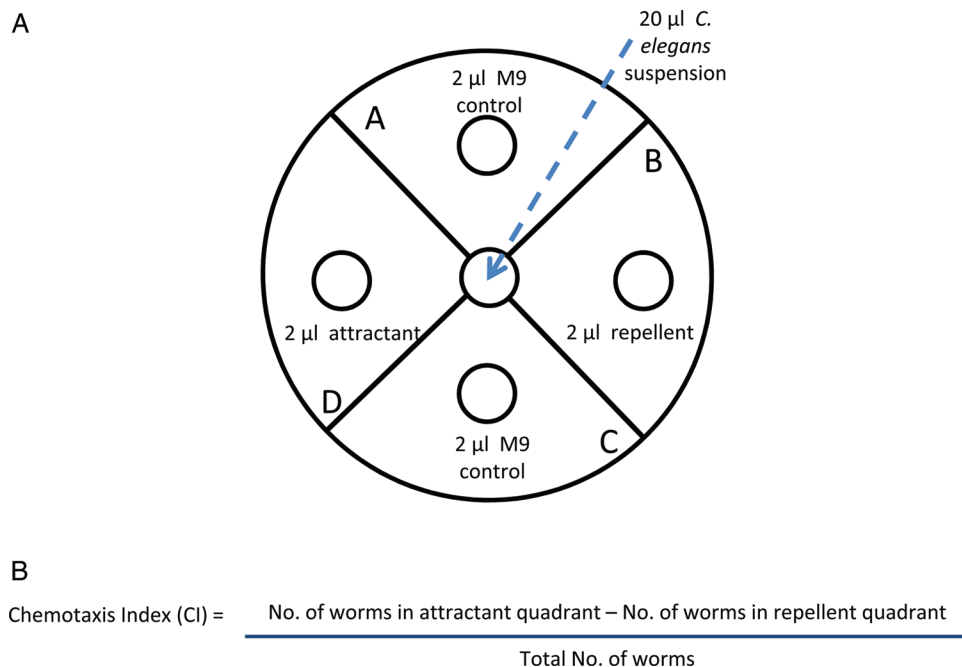


Figure 2. Chemosensory assay design and chemotaxis index (CI) equation. (A) Chemosensory assay plate design with 2 µl butanone + sodium azide as a chemoattractant (Quadrant D) and 2 µl SDS + sodium azide as a chemorepellent (Quadrant B). Two microlitre M9 buffer without sodium azide was used as a control in Quadrants A and C. Worm suspension was added to the centre circle as indicated. (B) Chemosensory response for each assay was calculated using the chemotaxis index equation.

Statistical analysis

Graphs were created showing mean values \pm SEM (Standard Error of the Mean) using Excel (2010). One-way, two-way and three-way analysis of variance (ANOVA) were performed using Minitab 17. Two sample *t*-tests were also performed using Minitab 17. An alpha level of <0.05 was considered statistically significant.

Results

The effects of hypoxia, LiCl and VA on chemosensory response of *C. elegans* were analysed using the chemosensory assay as described. CI was calculated for WT and HIF-1 mutant strains under both hypoxic and oxic conditions to determine chemosensory response. Normal chemosensory response was indicated by a high CI, and decreased chemosensory response was indicated by a low CI.

Initial analysis of the effects of hypoxia exposure on chemosensory behaviour in comparison to oxic conditions was carried out on WT and HIF-1 mutant strains. It was found that HIF-1 mutants were unable to adapt to hypoxia. LiCl and VA treatment was then introduced; the inability of HIF-1 mutants to adapt to hypoxic conditions was rescued by treatment with LiCl. Treatment with VA showed an impaired chemosensory response of both strains of *C. elegans*, independent of hypoxia.

HIF-1 is required for chemosensory adaptation to hypoxia in *C. elegans*

The results of the hypoxia chemosensory assay show that the WT *C. elegans* under oxic exposure showed the highest CI which was interpreted as normal chemosensory function (Fig. 3). The WT strain showed a mean CI value of between 0.6 and 0.7 under both oxic and hypoxic conditions; this shows that adaptation to hypoxia was taking place since the chemosensory response remained the same. The HIF-1 mutant was unable to adapt to hypoxic conditions, evident from a low CI value of <0.2 . This was significantly different from both the WT oxic group and the WT hypoxic group, indicating that HIF-1 is utilized in *C. elegans* in response to hypoxic conditions. The CI of HIF-1 mutants under oxic conditions was not significantly lower than the CI of WT under oxic conditions, suggesting that HIF-1 is not utilized in normal oxygen conditions.

LiCl exposure rescues chemosensory response to hypoxia in HIF-1 mutant *C. elegans*

Caenorhabditis elegans were treated with LiCl and VA to investigate the effects on chemosensory response with hypoxia exposure. A 48 h exposure time to pharmacological agents was chosen alongside the 48 h exposure to oxic or hypoxic conditions. A concentration of 3 mM LiCl and VA was chosen based on previous studies (Evason *et al.*, 2008; Leng *et al.*, 2008).

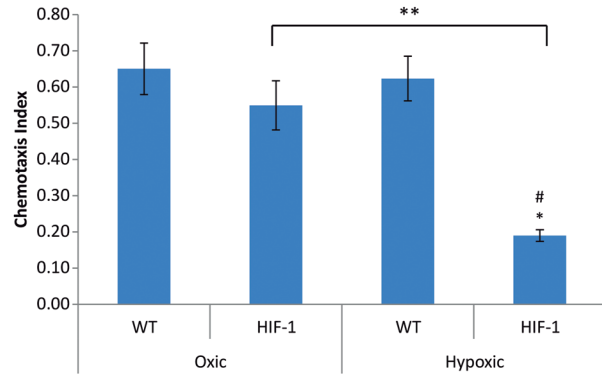


Figure 3. Chemosensory response of *Caenorhabditis elegans* in hypoxic and oxic conditions. WT and HIF-1 mutant *C. elegans* were exposed to hypoxic or oxic conditions for 48 h prior to chemosensory assays. The CI was calculated 60 min after starting the assays. Data represent the mean CI from five independent experiments, with each assay containing between 50 and 200 worms \pm SEM (* $p < 0.05$ against WT hypoxic control; # $p < 0.05$ against WT oxic control, ** $p < 0.05$ against oxic HIF-1 mutant group).

Exposure to 3 mM LiCl for 48 h prior to the chemosensory assays showed a significant rescue in CI in HIF-1 mutant strains under hypoxia compared with the controls (Fig. 4A). This demonstrated that HIF-1 mutant chemosensory response in 48 h of hypoxia exposure is restored with LiCl treatment.

Interestingly, exposure to VA alone in WT strains significantly decreased CI compared with the untreated control, indicating that this decline in chemosensory response is independent of hypoxia (Fig. 4B). A significant decrease in the CI of VA-treated HIF-1 mutants under hypoxia was also seen; this was expected based on the effects of VA on HIF-1 α expression previously mentioned. No significant difference in CI between VA-treated and untreated HIF-1 mutants under hypoxia was found. This could indicate that either the reduction in CI induced by hypoxia in HIF-1 mutants is not exacerbated further by VA treatment or that the CI value is at the lower limit and that any additional effects of VA are not showing on the CI scale. Using different concentrations of VA may assist in separating these theories and determining whether VA has the potential to decrease CI in HIF-1 mutants under hypoxia exposure further.

The CI of the WT strain under oxic conditions with the combined LiCl and VA treatment is significantly lower than the respective control (Fig. 4C). This indicates that the effects of LiCl and VA combined reduced chemosensory response, independent of hypoxia. This decrease in chemosensory response is most likely to be induced by the actions of VA, based on the results from the effects of VA treatment assay. This indicates that the actions of VA on chemosensory response of *C. elegans* are dominant over the actions seen with LiCl, which did not significantly alter the CI independent of hypoxia. Treatment with LiCl and VA on the HIF-1 mutant strain exposed to hypoxia did not show any significant increase or decrease in CI from the controls indicating

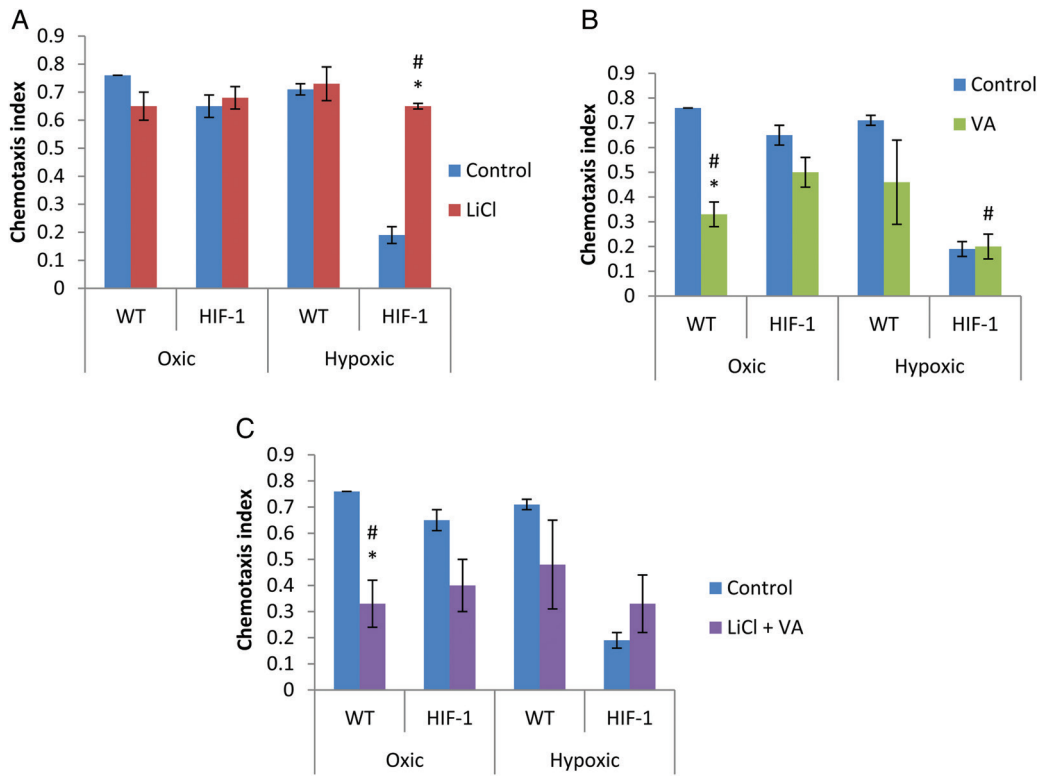


Figure 4. Chemosensory response of *Caenorhabditis elegans* in hypoxic and oxenic conditions with LiCl and VA treatment. WT and HIF-1 mutant *C. elegans* were exposed to hypoxic or oxenic conditions and treated with 3 mM LiCl and/or VA for 48 h prior to chemosensory assays. The CI was calculated 60 min after starting the assays. **(A)** CI of control (no drug treatment) vs. 3 mM LiCl-treated worms. **(B)** CI of control vs. 3 mM VA treated worms. **(C)** CI of control vs. 3 mM LiCl and 3 mM VA-treated worms. Data represent the mean CI from three independent experiments, with each assay containing between 50 and 200 worms \pm SEM (* $p < 0.05$ against respective exposure control; # $p < 0.05$ against WT oxenic control).

that the therapeutic actions of LiCl seen alone are being masked by the presence of VA. This again indicates a dominant effect of VA over LiCl. The error bars for LiCl and VA are larger than the other assays indicating that there was a considerable amount of variation within individual repeats; therefore, the data for the effects of LiCl and VA combined on chemosensory behaviour are inconclusive and require further investigations.

Discussion

The effect of hypoxia alone did not impair the chemosensory response of WT strains but did significantly impair the response of HIF-1 mutants; this indicates that HIF-1 mediates chemosensory response in hypoxia. A study by Chang and Bargmann (2008) demonstrated that WT *C. elegans* grown in hypoxic conditions for 48 h showed a lower preferred oxygen level than those grown in oxenic conditions. Oxygen preference which is normally influenced by the presence of food was switched off in WT strains cultured in hypoxic conditions, shown through the avoidance of bacterial food sources in hyperoxic conditions (21% O₂). HIF-1 mutants grown in hypoxic conditions did not avoid hyperoxia in the presence of

food, indicating that HIF-1 pathway mediates both behavioural and physiological changes in hypoxia.

Treatment with LiCl rescued the chemosensory response of HIF-1 mutants under hypoxia. This is in line with evidence that LiCl inhibits GSK-3 β rendering it inactive, and that this inhibition prevents GSK-3 β induced hydroxylation and proteasomal degradation of HIF-1 α (Chiu and Chuang, 2010). Ultimately, this results in an increased stability of HIF-1 α and the transcription of genes involved in adaptation to hypoxia (Mottet *et al.*, 2003). This pathway is conserved in *C. elegans*; therefore, it can be argued that LiCl increased stability of HIF-1 in hypoxia in the *C. elegans* HIF-1 mutant model. In addition to this study which has investigated hypoxia adaptation with LiCl, neuroprotective properties of LiCl have also been reported in a Huntington's disease model of *C. elegans* using a food clearance assay (Voisine *et al.*, 2007).

HIF-1 mutants treated with VA alongside hypoxia exposure showed a significantly lower CI compared with the WT oxenic control group. This is comparable to evidence which has shown that VA down-regulates HIF-1 expression through the inhibition of HDACs (Machado-Vieira *et al.*, 2011). VA did not significantly impair the adaptation to hypoxia in WT

strains, suggesting a differential effect of VA on WT and HIF-1 mutant strains. VA significantly decreased the chemosensory response of WT strains in comparison to untreated controls, independent of hypoxia.

This effect suggests that VA has a detrimental effect on the neuronal function of *C. elegans* in oxic conditions, in addition to hypoxic conditions. A study by Cipro *et al.* (2012) investigated the effect of VA *in vitro* on neuroblastoma cell lines with hypoxia exposure. They observed a similar effect with VA treatment, an increase in apoptotic activity of cells was seen in both oxic and hypoxic conditions compared with controls indicating that VA can act independently of hypoxia. These results suggest that downstream targets of VA have an inhibitory effect on the chemosensory response. Further research is required to identify the mechanisms of chemosensory behaviour impairment seen with VA in *C. elegans*. It would be interesting to investigate further using inhibitors of potential downstream targets which alter the neuronal function of *C. elegans*. Alternatively, it could be that the concentration of VA used was high enough to elicit a toxic effect and so the effects of different concentrations of VA should also be investigated.

The effects of combined LiCl and VA treatment on *C. elegans* in hypoxia have not previously been investigated. VA appeared to elicit a dominant effect over LiCl which resulted in a significant decrease in chemosensory response, independent of hypoxia. It would be interesting to take these investigations further by using different assays to analyse the effects of LiCl with VA on motor behaviour and feeding behaviour of *C. elegans*, with hypoxia.

Conclusion

HIF-1 has previously been identified as an essential transcription factor that modulates oxygen homeostasis in *C. elegans* throughout their lifespan (Zhang *et al.*, 2009). *In vivo* analysis of *C. elegans* in this study showed that the chemosensory response of HIF-1 mutant strains was significantly impaired upon hypoxia exposure; this strongly indicates that HIF-1 is necessary for adequate chemosensory response in *C. elegans* under hypoxic conditions. The impaired chemosensory response was rescued by treatment with LiCl, indicating that LiCl had a stabilizing effect on HIF-1 in *C. elegans* allowing adaptation to hypoxia. VA treatment resulted in a decreased chemosensory response of *C. elegans* independent of hypoxia. VA treatment also resulted in a decreased chemosensory response with hypoxia exposure. Interestingly, VA treatment has been reported to extend the lifespan of *C. elegans* in a study by Evason *et al.* (2008); however, this study did not include any exposure to hypoxia conditions. This study also reported no effects on *C. elegans* during development stages, but a delay in degeneration was observed, which may have resulted from the formation of dauer worms which can survive for longer in undesirable conditions. It would be interesting to investigate the effects of VA treatment on lifespan

further but with exposure to fluctuating oxygen conditions during development. LiCl treatment has also been implicated in extending the lifespan of *C. elegans* (Tam *et al.*, 2014), again this would be interesting to investigate further with HIF-1 mutants and exposure to fluctuating oxygen levels. Data from LiCl and VA treatment combined were inconclusive, to investigate this further a *C. elegans* EGL-9 mutant could be used. Interactions of LiCl and VA alone and combined with the negative regulator EGL-9 could be investigated with exposure to hypoxia. LiCl has been associated with neuroprotective properties, and the evidence from the *in vivo* results strongly indicates that it has a protective effect on the chemosensory behaviour of HIF-1 mutants under hypoxia.

Authors' biography

G.M. graduated from the University of Bath in 2014 with a distinction in MSc Medical Biosciences. Prior to this, she graduated with an Honours degree in BSc Biological Sciences at the University of Leeds. Her interests lie in neuroscience and cancer biology.

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