Published in final edited form as:

Respir Physiol Neurobiol. 2014 September 15; 201: 24–30. doi:10.1016/j.resp.2014.05.012.

Oxygen related chemoreceptor drive to breathe during H₂S infusion

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Abstract

This study addresses the following question: Could the acute depression in breathing produced by hyperoxia, a reflection of the tonic drive to breathe from the arterial chemoreceptors, be accounted for by the presence a background level of local endogenous H₂S? To address this question, we produced a stable but moderate increase in breathing ($24 \pm 11\%$) via continuous infusion of low levels of H₂S, in 10 spontaneously breathing urethane-sedated rats. We found that acute exposure to 100% O₂ (20 tests) decreased minute ventilation (V_I) from 301 ± 51 to 210 ± 43 ml/min within 15 seconds in control conditions, but no additional significant drop in V_I was observed during H₂S induced hyperpnea. In addition, no decrease in the estimated concentrations of gaseous H₂S in the arterial blood was observed during the hyperoxic tests. It is concluded that the ventilatory depression induced by high O₂ appears to be limited to the tonic background peripheral chemosensory drive to breathe, but has little or no impact on the CB stimulation produced by low levels of H₂S.

1. Introduction

When abruptly exposed to pure O₂, mammals displayed an immediate reduction in breathing by 10–30%, a result of the inhibition of the carotid body tonic activity (Dejours, 1962; Dejours et al., 1957; Leitner et al., 1964; Leitner et al., 1965). This response is exaggerated when the background level of the CB activity is high as during hypoxia (Dejours, 1962; Dejours et al., 1957; Leitner et al., 1964; Leitner et al., 1965). Following the original description of this test by Dejours in the late 50's (Dejours, 1962; Dejours et al., 1957; Leitner et al., 1964; Leitner et al., 1965), the fast component of the depressive response to oxygen has been become a standard test to evaluate the contribution of the CB to the drive to breathe or at least to demonstrate the presence of such a drive. The mechanism(s), explaining why and how some CB cells are excited at normal PaO₂ and would require high levels of O₂ (above 150–200 Torr) to be silenced, remains unclear. In this study we have

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tried to determine whether H₂S that could be present endogenously in the carotid body (Peng et al., 2010) could account for this background activity. Indeed, it has been proposed that 1- H₂S is produced and is present in baseline conditions in the chemoreceptor cells, 2- H₂S concentration could increase in hypoxia (Peng et al., 2010) possibly via a decrease in H₂S rate of oxidation (Olson, 2008). Finally, since the application of exogenous H₂S results in a stimulation of CB cells in vitro (Peng et al., 2010), as long been shown in vivo ((Haouzi, 2012) for review), H₂S has been proposed to transduce the effects of hypoxia. The argument of causality between the response of the CB to hypoxia and an increase in endogenous H₂S relies on the observation that the response to hypoxia is blunted after inhibiting the cystathionine gamma-lyase (CSE), one of the enzymes responsible of the formation of H₂S in the CB, or in CSE deficient mice (Olson, 2011; Peng et al., 2010). If we assume that there is a baseline level of H₂S in the CB, could the presence of this molecule contribute to the background activity of the CB at ambient P_{O₂}?

The rational we used in the present study could be summarized as follows: (1) abrupt exposure to hyperoxia suppresses the tonic drive to breath, (2) H₂S, if present in the CB is oxidized by the mitochondria requiring O₂ in the process and its concentration produced immediate changes in CB activity, (3) increasing local PO₂ in the CB cells can increase/enhance H₂S “oxidation”; this proposition is however only valid if H₂S oxidation is O₂ limited during air breathing or moderate hypoxia. We have tried to quantify the magnitude of the decrease in breathing produced by acute hyperoxia, during H₂S induced hypernea obtained by infusing low dose of exogenous H₂S. The latter is assumed to increase H₂S concentrations in the CB, which in turn produces a moderate increase in breathing. Our working hypothesis is that If, at a given H₂S partial pressure, hyperoxia is able to reduce the component of H₂S induced breathing stimulation, this will indicate that H₂S can be oxidized in CB cells via an O₂ limited mechanism. In other words, although it will remain to be shown that H₂S is actually present in the CB at a concentration capable of stimulating CB, the effects of hyperoxia on H₂S induced breathing stimulation will help us to clarify whether the characteristics of a model linking O₂ sensing and H₂S could operate through H₂S oxidation in the presence of oxygen (Peng et al., 2010).

2. Material and Methods

2.1. Animal preparation

Ten adult male Sprague-Dawley rats weighing 571 ± 33 g were studied. All experiments were conducted in accordance with the Guideline for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85–23, revised 1996). The study was approved by the Pennsylvania State University College of Medicine Institutional Care and Use Committee. Anesthesia was initiated with 3.5% isoflurane in O₂ followed by a unique injection of urethane (1.2 g/kg, intraperitoneal). Then, the rats were tracheostomized (14G surflo catheter, Terumo, NJ) as previously described (Haouzi and Van de Louw, 2013). A catheter was inserted into the right femoral artery for systemic blood pressure monitoring and arterial blood sampling (PE-50 tubing) while an additional catheter was inserted into the right femoral vein for infusion of H₂S. Body temperature was monitored with a rectal probe (Thermalert TH-5, Physitemp, Clifton NJ)

and maintained at around 36.5°C with a heating pad and lamp. At the end of the experiment, rats were euthanized with CO₂ followed by loading dose of pentobarbital (200 mg/kg) and aortic dissection.

2.2 Measurements and data analysis

The tracheal catheter was connected to a low dead space 2-way valve (Hans Rudolph, KS) and the inspiratory flow was measured using a pneumotachograph (1100 Series, Hans Rudolph, Shawnee, KS) (Haouzi and Van de Louw, 2013). The experimental dead space corresponding to the valve chamber and the T piece between the trachea and the valves was 1.2 ml. The inspiratory valve could be connected to bags containing various fractions of O₂ or CO₂, the circuit was designed so it could allow to switch any new gas mixture at the level of the valve, changing within one breath the composition of the inspired gas. The expiratory valve was connected to a series of two 5 ml mixing chambers (Klingerman et al., 2013). An additional source of air was introduced into the first mixing chamber in which flow was continuously measured using another pneumotachograph (1100 Series, Hans Rudolph, Shawnee, KS). Mixed expired H₂S fractions were measured continuously from the second mixing chamber using H₂S analyzer (Interscan RM series, Simi Valley, CA; range: 0.001–1 ppm). The arterial catheter was connected to a pressure transducer (TA-100, CWE Inc., Ardmore, PA). All signals were digitized at 200 Hz using an analog-to-digital data acquisition system (Power Lab 16/35, AD Instruments, Colorado Springs, CO) and stored for later analysis. Breathing frequency (f) and tidal volume (V_T) were determined using peak detection and integration of the inspiratory flow signal, respectively, and minute ventilation (V_I) was computed as f. V_T in BTPS conditions (Klingerman et al., 2013). Arterial blood gases partial pressures in O₂ (PaO₂) and CO₂ (PaCO₂) were measured using i-STAT1 blood gas analyzer (ABAXIS, Union city, CA).

2.3. Measurement of dissolved H₂S in the arterial blood

H₂S was administered intravenously as solution of sodium hydrosulfide hydrate (20 mM NaHS, Sigma Aldrich, St Louis, MO) in sterile physiological saline, prepared immediately prior each experiment and kept in airtight syringes. H₂S was infused using a syringe pump (Fusion 100, Chemyx Inc., Stafford, TX) via catheter in femoral vein at a rate of 0.30 to 0.50 ml/min (7.0 ± 1.0 μmol/min). This level was selected as it stimulates breathing with no risk of apnea or drop in blood pressure (Klingerman et al., 2013). The fraction of H₂S was continuously measured from the second mixing chamber; then mixed, expired H₂S fraction was computed to calculate the partial pressure of alveolar H₂S (FA_{H₂S}) as previously described (Klingerman et al., 2013). The concentration of gaseous H₂S in the blood (C_{gH₂S}) was then calculated assuming a coefficient of solubility of 0.00012 mol/l/mmHg (Douabul and Riley, 1979; Klingerman et al., 2013).

2.4. Experimental protocols and data collection

Following 10 min of stable spontaneous breathing in room air, rats were exposed to 10% O₂, balance N₂, for 90 s. The rats were allowed a recovery period of 10 min then were exposed to 100% O₂ with or without 3% CO₂ for 90 s. In the latter 97% O₂-3% CO₂ was used, following 3 minutes of breathing 3% CO₂ in air. As developed in the result section our goal was to appreciate the depressive effects of hypocapnia during sulfide infusion on the

magnitude of the response to hyperoxia. The choice of 3 % CO₂ was dictated by our estimate of how much PaCO₂ may increase in keeping with the absolute level of CO₂ production, the total dead-space with our experiment setting (see discussion section).

After recovery from hyperoxic exposure, H₂S was infused during air breathing or air with 3 % CO₂. Three minutes into infusion, new hyperoxic tests were applied as described above. Arterial blood samples (100 µl) were collected before and at the end of hyperoxic exposure. Every test was repeated twice in the same rat in a random manner; data from each 2 sets of tests were averaged for statistical analysis. Baseline minute ventilation was averaged over 30 s before each hyperoxic test and every 5 seconds following the onset of hyperoxic exposure. The lowest value of minute ventilation occurring during the first 20 seconds of hyperoxic exposure was regarded as the nadir of ventilatory depression.

2.5. Rationale and anticipated results

Exposing rats to O₂ will abruptly suppress the chemoreceptor drive to breath and drop breathing within seconds. If this mechanism relates to the rate of H₂S oxidation, then during H₂S induced hyperpnea, exposure to O₂ should also depress the component of breathing stimulation produced by sulfide. If H₂S in the CB is not affected by O₂ exposure, we should still expect a decrease in ventilation related to the chemoreceptor drive to breath (figure 1B), but the lack of effect of O₂ on H₂S induced hyperpnea may indicate that H₂S acts through a parallel mechanism on CB function and that H₂S oxidation is not O₂ limited during air breathing or during moderate hypoxemia.

2.6. Data analysis and Statistical Analysis

All statistical analyses were conducted using GraphPad Prism 5 (Graphpad Software, La Jolla, CA, USA). Data are expressed as means ± SD. The effect of hyperoxia was analyzed as follows: paired-t test was used for comparison of minute ventilation before and at the nadir of ventilatory depression in hyperoxia. One-way repeated ANOVA and Bonferroni's post hoc test were used for comparison of ventilation among the various conditions (hyperoxia with or without NaHS solution and with or without 3% CO₂); data were compared in absolute value, delta changes and % of change. Significance was accepted at P<0.05.

3. Results

3.1. Response to acute hyperoxia

Figure 1 illustrates the effects of an exposure to 100% O₂ on minute ventilation in one rat, while the averaged responses (20 tests in 10 animals) are displayed in Figure 2. All ventilatory and PaCO₂ values are also summarized in table 1. The baseline level of minute ventilation was 301 ± 51 ml/min (Fig. 2a) with a tidal volume of 2.4 ± 0.4 ml and a frequency which averaged 126 ± 15 c/min. Switching to 100% O₂ increased arterial PaO₂ from 68 ± 9 to 253 ± 100 Torr (P<0.001). This was associated with an immediate reduction in minute ventilation to 279 ± 44 ml/min (−7 ± 3%, P<0.01) at 10 seconds of exposure. The nadir was reached at 15 seconds and averaged 210 ± 43 ml/min (−30 ± 6%, P<0.001, Fig.

2a). This reduction in breathing led to a rise in PaCO_2 from 37 ± 4 to 46 ± 3 mmHg, $P < 0.001$). $\text{FA}_{\text{H}_2\text{S}}$ was undetectable.

To determine the influence of the level of PaCO_2 on the magnitude of response to O_2 test in our model, the same procedure was repeated, while the rats were breathing 3% CO_2 in air, for 5 minutes prior to and during the Dejours test. After adding CO_2 into the inspired gas V_I increased significantly (338 ± 61 ml/min, $+12 \pm 8\%$, $P < 0.01$), tidal volume reached 2.7 ± 0.5 ml with a frequency of 125 ± 19 c/min. PaCO_2 rose with large variability between animals by about 3 Torr (from 37 ± 4 to 40 ± 5 Torr). Abrupt exposure to O_2 produced a significant reduction in minute ventilation (266 ± 39 ml/min, $P < 0.001$), but V_I remained significantly higher than during the control hyperoxic test ($P < 0.001$). In other words, the ventilatory depression triggered by pure O_2 was not significantly enhanced by the presence of 3% CO_2 .

3.2. Acute response to hyperoxia during H_2S infusion in poikilocapnic conditions

As shown in Figure 3, H_2S infusion increased minute ventilation, reaching a steady state within about one minute. V_I rose from 308 ± 54 to 379 ± 48 ml/min 3 minutes into H_2S infusion ($24 \pm 11\%$ increase, $P < 0.001$, Fig. 2a). This increase in breathing was associated to a decrease in PaCO_2 (from 37 ± 4 to 32 ± 5 Torr, $P < 0.05$, Fig. 2c). $\text{FA}_{\text{H}_2\text{S}}$ increased during H_2S infusion reaching 13.7 ± 5.8 ppm, corresponding to an arterial partial pressure of H_2S of $10 \pm 4 \cdot 10^{-3}$ Torr or a concentration of gaseous H_2S in the arterial blood ($\text{C}_{\text{gH}_2\text{S}}$) that averaged 1.25 ± 0.53 μM . Abrupt exposure to hyperoxia increased PaO_2 from 77 ± 7 to 223 ± 76 Torr ($P < 0.001$) and was associated with a decrease in minute ventilation (339 ± 47 ml/min) during the first 10 seconds of exposure ($-11 \pm 2\%$, $P < 0.001$). As shown in Figure 2A and table 1, minute ventilation reached a nadir of 254 ± 44 ml/min ($-33 \pm 7\%$, $P < 0.001$) within 15 seconds, that was significantly larger than in control hyperoxic condition (Figure 2C, $P < 0.001$) but was still higher than baseline V_I (Figure 2B). The absolute level of ventilation during the Dejours test and H_2S infusion was higher than that in control hyperoxic conditions (Figure 2A). As a result PaCO_2 during hyperoxic tests was significantly lower than in control hyperoxic conditions (38 ± 6 Torr, $P < 0.01$, Figure 2D). H_2S concentration was not depressed during hyperoxic exposure ($\text{C}_{\text{gH}_2\text{S}} = 1.38 \pm 0.55$ μM , Figure 2E).

3.3. Acute response to hyperoxia during H_2S infusion with 3% CO_2 background

H_2S infusion with a 3% CO_2 background increased V_I to 411 ± 56 ml/min (Figure 2A). Despite the addition of CO_2 , PaCO_2 was still lower than in control conditions during H_2S infusion (33 ± 2 vs. 37 ± 4 Torr in control rats, $P < 0.05$). V_I decreased to 380 ± 56 ml/min ($-8 \pm 4\%$) within 10 seconds of O_2 exposure, and reached its nadir (308 ± 50 ml/min, $-25 \pm 7\%$, $P < 0.001$, Figure 2A) within 15 seconds. As shown in figure 2 and table 1, the absolute level of ventilation was significantly higher than during hyperoxia in control condition ($P < 0.001$), and was not different from baseline, i.e. prior to H_2S infusion (Figure 2C), the change in ventilation was not different from control hyperoxic test (Figure 2B) despite a lower PaCO_2 (41 ± 7 Torr, Figure 2C). In other words, hyperoxia preserved the component of breathing stimulation induced by sulfide which remained at 308 ± 50 ml/min, at the nadir of the response to O_2 (with 3% CO_2), vs. 301 ± 51 ml/min in baseline control conditions

(see also table). C_{gH_2S} averaged $1.22 \pm 0.59 \mu M$ during H_2S exposure and did not decrease during hyperoxia (C_{gH_2S} , $1.47 \pm 0.92 \mu M$) as shown on Figure 2E.

3.4. 10% hypoxia in spontaneous breathing rats

Every animal, with no exception, displayed a clear ventilatory stimulation when exposed to 10% O_2 (Figure 3). Minute ventilation increased from 291 ± 33 ml/min to 456 ± 53 ml/min during 10% O_2 ($58 \pm 17\%$, $P < 0.001$), which was if anything higher than during H_2S infusion ($P < 0.001$). The hyperventilation was associated, in both conditions, with an increase in the production of augmented breaths. This results indicate that the level of stimulation produced by H_2S in our study would be similar to level of FIO_2 higher than 10%.

3.5. Additional observation

The sulfide solution was prepared in saline and had a pH of 9.84. During H_2S infusion without any CO_2 added, the only change in arterial blood gas composition was a respiratory alkalosis with a concomitant reduction in bicarbonate level: $pH = 7.41 \pm 0.03$, $HCO_3^- = 19.8 \pm 1.7$ meq/l and $PaCO_2 = 32 \pm 5$ Torr during H_2S infusion, compared to $pH = 7.28 \pm 0.02$, $HCO_3^- = 21.8 \pm 1.79$ meq/l, and $PaCO_2 = 37 \pm 4$ Torr in control conditions. This effect was obviously less pronounced during CO_2 inhalation with H_2S infusion wherein $pH = 7.39 \pm 0.03$, $HCO_3^- = 20.1 \pm 2.5$ meq/l, and $PaCO_2 = 37 \pm 4$ Torr. In other words, there was no evidence of any metabolic alkalosis during H_2S infusion related to the pH of the solution, which could have affected the arterial chemoreflex.

4. Discussion

We found that an abrupt exposure to pure O_2 had a small or no depressive effect on the ventilatory stimulation produced by an infusion of exogenous H_2S . This effect was not even visible when hypocapnia secondary to H_2S induced hyperpnea was partially corrected. The effects of hyperoxia were studied during moderate H_2S exposure, able to increase breathing by about 20 %. For the sake of comparison, such a level of stimulation was even more modest than what was produced by 10% hypoxia (Figure 3).

4-1 H_2S and hyperoxia effects on breathing

Intravenous bolus injection of an exogenous solution containing hydrogen sulfide (H_2S/HS^-) has long been shown to increase breathing (Heymans et al., 1933; Winder and Winder, 1933), along with a production of augmented breaths (Klingerman et al., 2013), which results from the stimulation of the peripheral chemoreceptors. Medullary effects of H_2S/HS^- consist in a depression of breathing (Greer et al., 1995; Klingerman et al., 2013). More recently Chen et al. (Chen et al., 2013) have reported some increase in discharge rate of respiratory neurons when applying concentrated solution of H_2S directly on isolated medullary structures. The “respiratory” effects, which were very modest, affected mostly breathing frequency and occurred with a long delay and time constant (several minutes), and are therefore difficult to reconcile to the immediate and brisk increase in tidal volume together with the production of sighs, observed in vivo and typical of CB stimulation.

Hyperoxia has long been shown to depress with no delay the background neural activity in the sinus nerve along with breathing (Leitner et al., 1964; Leitner et al., 1965). Interestingly, the highest the level of stimulation (hypoxia) prior to a hyperoxic test, the largest the ventilatory depression (Dejours, 1962; Dejours et al., 1957). Clearly the approach proposed by Dejours (Dejours, 1962), wherein the ventilatory depression provoked by an abrupt increase in PaO_2 is enhanced by prior-hypoxic condition (Zapata et al., 2012), was unable to significantly depress the component of H_2S -induced ventilatory stimulation.

How to reconcile the present results with our previous data suggesting that the response to H_2S is blunted against a background of hyperoxia (Haouzi et al., 2011)? We have recently found that the ventilatory response to a bolus injection of H_2S was reduced under hyperoxic conditions (100% O_2) in the rat (Van de Louw and Haouzi, 2012) and in the sheep (Haouzi et al., 2011). We postulated that a rapid oxidation of H_2S by “pure” O_2 might have occurred during H_2S transit time in the pulmonary capillary bed, the pulmonary venous system or the arterial blood, before reaching the CB chemoreceptors. However, we also found that H_2S added in vitro to blood was not affected by PO_2 ranging from 23 to 760 Torr (Van de Louw and Haouzi, 2012), consistent with our present findings that, in vivo, the level of dissolved H_2S in the blood is not decreased by hyperoxia, at least within few tens of seconds. In our previous studies, “prolonged” exposure to pure O_2 , i.e. for more several minutes, prior to H_2S injection could have affected the production of reactive O_2 species as well as NO metabolism, which in turn may interact with the potential effects of H_2S (Coletta et al., 2012; Kajimura et al., 2010) and/or the gain of the arterial chemoreflex (Haxhiu et al., 1995; Prabhakar et al., 1993; Wang et al., 1994), regardless of local H_2S concentrations. Finally, it is difficult to compare the effects of a bolus injection of H_2S , which will produce a very transient effects, to that of a continuous infusion.

Buckler and Turner (Buckler and Turner, 2013) have recently shown that the electro-chemical gradient of the mitochondrial membrane of chemoreceptor cells can be lowered by reducing O_2 supply to levels corresponding to moderate hypoxia. The exact mechanisms underlying such an exquisite sensitivity of the mitochondria in the carotid bodies (CB) to a decrease in O_2 remains to be understood (Lahiri et al., 2001; Peers et al., 2010). This observation has also implications relevant to the debate on the role of sulfide in the CB. Indeed, in another study, Buckler (Buckler, 2012) reported that a concentration as low as 7.5 microM, applied on CB cells, depresses the mitochondrial function, just like moderate hypoxia. Taking into account the evaporation factor (Deleon et al., 2011), these results suggest that the mitochondrial activity in CB cells may well be affected at lower doses than previously reported, i.e 30 micromolar solution of exogenous H_2S appears to be required to excite type-1 CB cells in-vitro (Peng et al., 2010). These concentrations are toxic for the cytochrome c oxidase activity, inhibiting the mitochondrial function of CB cells (Buckler, 2012) and of many other cellular systems (Bouillaud and Blachier, 2011; Cooper and Brown, 2008). The levels of H_2S stimulating the CB reported by Buckler (Buckler, 2012) are therefore not so different from the in vivo levels of *gaseous* H_2S we found associated with a stimulation of breathing during sulfide infusion-about 1 microM of gaseous H_2S , 4 microM including HS^- or 10 microM for all sulfide present including in combined forms (Klingerman et al., 2013).

In keeping with the mitochondrial hypothesis, although it is not known whether at ambient P_{O_2} the baseline mitochondrial membrane in CB cells could be even “more” polarized by pure O_2 resulting in a higher shift of protons and increase in ATP production, the background level of mitochondrial depolarization can certainly affect the response to hyperoxia. Whenever H_2S is present in the CB, its affinity for the cytochrome c oxidase could prevent high O_2 to express its own effect on the mitochondrial membrane potential. Conversely, it is possible that in the presence of a hyperoxic background, higher level of sulfide than during air breathing would be required to depolarize the mitochondrial membrane. Investigating such a mechanism has important implications for our understanding on the nature of the oxygen drive to breathe at ambient P_{aO_2} .

4-2 Methodological limits

Several methodological issues should be considered. First the approach used to estimate CgH_2S was based on the determination of the expired fraction of H_2S as previously described (Klingerman et al., 2013); the accuracy of this method as well as the effects of PI_{CO_2} on PA_{CO_2} depend on the difference between minute ventilation and alveolar ventilation. Indeed, in baseline condition, we found that Pa_{CO_2} averaged 37 mmHg, while minute ventilation=295 ml/min, $V_T=2.34$ ml and $f=126.\text{min}^{-1}$. We have in previous studies measured V_{O_2} in a similar animal model (Haouzi and Van de Louw, 2013), values of V_{CO_2} around 4 ml/min are not unexpected in our sedated rats. In keeping with the relationship between V_{CO_2} and dead space ($KV_{CO_2} = (VE-VD) \cdot Pa_{CO_2}$ eq 1), it is possible to estimate VD. Based on this relationship, VD should average 1.6 ml (including 1.2 ml corresponding to the instrumental dead space) in keeping with the baseline level of minute ventilation and PA_{CO_2} . Increasing PI_{CO_2} by 20 Torr at the level of ventilation obtained during CO_2 breathing (340 ml/min, $V_T=2.72$ ml and $f=125.\text{min}^{-1}$) and using the above estimated value of dead space and V_{CO_2} would lead to an average of Pa_{CO_2} of 42–43 mmHg. We found that Pa_{CO_2} during 3% CO_2 breathing was 2 Torr less than what this model would have predicted. The most significant sources of error we could identify include our initial estimation of V_{CO_2} , the determination of Pa_{CO_2} which resolution is no better than one Torr. It is also possible that 3 minutes of exposure of 3% CO_2 were not long enough to reach a steady state. Although isocapnia was not maintained while inhaling 3% CO_2 , we could still establish that hypocapnia produces, if anything, a magnification of the response to hyperoxia. So an even lower depression in breathing is to be anticipated if Pa_{CO_2} were kept at higher levels.

The model of urethane-sedated rats allows breathing control to be studied during air breathing but leads to levels of PaO_2 lower than what would be expected in unanaesthetized animals. Resting PaO_2 averaged 68 Torr in our rats; a level which has little effects on the CB function which discharge rate significantly increases at levels of PO_2 below 50 Torr. Urethane sedated rats have a ventilatory control system not very different from unanaesthetized animals except for a blunted production of augmented breathes.

4-3 Conclusions

Our working hypothesis was that, assuming that H_2S is present with a concentration capable of stimulating the CB, sulfide may contribute to the CB tonic drive to breath, if H_2S oxidation is O_2 limited at ambient PO_2 . Our results indicates that either the model used for

linking H₂S oxidation to O₂ sensing must be redefined, or that H₂S does stimulate the CB but using a pathway that may still be similar to the one used by hypoxia, but with no causal link between the 2 gases.

In conclusion, acute hyperoxia, which depresses the basal chemoreceptor drive to breathe in our rat models did not affect significantly H₂S-induced CB stimulation. This result does not support the hypothesis that baseline H₂S contributes to the tonic peripheral chemical drive to breath.

Acknowledgments

This work has been supported in part by the CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Grant Number 1R21NS080788-01. Takashi Sonobe is a researcher in the Department of Cardiac Physiology, National Cerebral and Cardio-vascular Center, Osaka, Japan, and is currently supported by a “Grant-in-Aid” for scientific research (N11J10987) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- Agapito MT, Sanz-Alfayate G, Gomez-Nino A, Gonzalez C, Obeso A. General redox environment and carotid body chemoreceptor function. *American journal of physiology. Cell physiology*. 2009; 296:C620–631. [PubMed: 19144860]
- Awata S, Nakayama K, Kodama H. Effect of D,L-propargylglycine on cystathionine metabolism in rats. *Biochemistry international*. 1984; 8:171–179. [PubMed: 6477595]
- Bouillaud F, Blachier F. Mitochondria and sulfide: a very old story of poisoning, feeding, and signaling? *Antioxidants & redox signaling*. 2011; 15:379–391. [PubMed: 21028947]
- Buckler KJ. Effects of exogenous hydrogen sulphide on calcium signalling, background (TASK) K channel activity and mitochondrial function in chemoreceptor cells. *Pflugers Archiv : European journal of physiology*. 2012; 463:743–754. [PubMed: 22419174]
- Buckler KJ, Turner PJ. Oxygen sensitivity of mitochondrial function in rat arterial chemoreceptor cells. *The Journal of physiology*. 2013; 591:3549–3563. [PubMed: 23671162]
- Chen L, Zhang J, Ding Y, Li H, Nie L, Zhou H, Tang Y, Zheng Y. Site-specific hydrogen sulfide-mediated central regulation of respiratory rhythm in medullary slices of neonatal rats. *Neuroscience*. 2013; 233:118–126. [PubMed: 23291458]
- Coletta C, Papapetropoulos A, Erdelyi K, Olah G, Modis K, Panopoulos P, Asimakopoulou A, Gero D, Sharina I, Martin E, Szabo C. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:9161–9166. [PubMed: 22570497]
- Cooper CE, Brown GC. The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *J Bioenerg Biomembr*. 2008; 40:533–539. [PubMed: 18839291]
- Dejours P. Chemoreflexes in breathing. *Physiological Reviews*. 1962; 42:335–358. [PubMed: 13884932]
- Dejours P, Labrousse Y, Raynaud J, Teillac A. Stimulus oxygene chemoreflexe de la ventilation a basse altitude (50 m) chez l’homme - I au repos. *Journal de Physiologie*. 1957; 49:115–119. [PubMed: 13449758]
- Deleon ER, Stoy GF, Olson KR. Passive loss of hydrogen sulfide in biological experiments. *Anal Biochem*. 2011
- Douabul AA, Riley JP. Solubility of Gases in Distilled Water and Seawater .5. Hydrogen-Sulfide. *Deep-Sea Research Part a-Oceanographic Research Papers*. 1979; 26:259–268.
- Greer JJ, Reiffenstein RJ, Almeida AF, Carter JE. Sulfide-induced perturbations of the neuronal mechanisms controlling breathing in rats. *Journal of applied physiology*. 1995; 78:433–440. [PubMed: 7759410]

- Haouzi P. Ventilatory and metabolic effects of exogenous hydrogen sulfide. *Respir Physiol Neurobiol*. 2012; 184:170–177. [PubMed: 22579639]
- Haouzi P, Bell H, Philmon M. Hydrogen sulfide oxidation and the arterial chemoreflex: effect of methemoglobin. *Respiratory physiology & neurobiology*. 2011; 177:273–283. [PubMed: 21569867]
- Haouzi P, Van de Louw A. Uncoupling mitochondrial activity maintains body [Formula: see text] during hemorrhage-induced O₂ deficit in the anesthetized rat. *Respiratory physiology & neurobiology*. 2013; 186:87–94. [PubMed: 23333818]
- Haxhiu MA, Chang CH, Dreshaj IA, Erokwu B, Prabhakar NR, Cherniack NS. Nitric oxide and ventilatory response to hypoxia. *Respiration physiology*. 1995; 101:257–266. [PubMed: 8606998]
- Heymans, C.; Bouckaert, JPR. Pression endovasculaire au niveau des zones vasosensibles cardio-aortique et sino-carotidiennes et regulation reflexe de l'activite du centre respiratoire. Doin et Cie G; Paris: 1933.
- Kajimura M, Fukuda R, Bateman RM, Yamamoto T, Suematsu M. Interactions of multiple gas-transducing systems: hallmarks and uncertainties of CO, NO, and H₂S gas biology. *Antioxidants & redox signaling*. 2010; 13:157–192. [PubMed: 19939208]
- Kamoun P. Endogenous production of hydrogen sulfide in mammals. *Amino Acids*. 2004a; 26:243–254. [PubMed: 15221504]
- Kamoun P. H₂S, a new neuromodulator. *Med Sci (Paris)*. 2004b; 20:697–700. [PubMed: 15329822]
- Klingerman CM, Trushin N, Prokopczyk B, Haouzi P. H₂s Concentrations in the Arterial Blood during H₂s Administration in Relation to Its Toxicity and Effects on Breathing. *American journal of physiology. Regulatory, integrative and comparative physiology*. 2013
- Kodama H, Ikeda H, Awata S, Nakayama K. Cystathionine accumulation in various regions of brain of DL-propargylglycine-treated rats. *J Neurochem*. 1985; 44(4):1207–1209. [PubMed: 3973610]
- Lahiri S, Rozanov C, Roy A, Storey B, Buerk DG. Regulation of oxygen sensing in peripheral arterial chemoreceptors. *Int J Biochem Cell Biol*. 2001; 33:755–774. [PubMed: 11404180]
- Leitner LM, Pages B, Dejourn P. Parallel Variations in Ventilation and Glomus Discharges During Brief Inhalations of Pure Oxygen in the Cat. *J Physiol (Paris)*. 1964; 56:390. [PubMed: 14219815]
- Leitner LM, Pages B, Puccinelli R, Dejourn P. Simultaneous study of ventilation and glomus caroticum chemoreceptor impulses in cats. II. During brief inhalations of carbon dioxide. *Arch Int Pharmacodyn Ther*. 1965; 154:427–433. [PubMed: 5838348]
- Lertratanangkoon K, Scimeca JM, Wei JN. Inhibition of glutathione synthesis with propargylglycine enhances N-acetylmethionine protection and methylation in bromobenzene-treated Syrian hamsters. *The Journal of nutrition*. 1999; 129:649–656. [PubMed: 10082769]
- Leschelle X, Gubern M, Andriamihaja M, Blottiere HM, Couplan E, Gonzalez-Barroso MD, Petit C, Pagniez A, Chaumontet C, Mignotte B, Bouillaud F, Blachier F. Adaptive metabolic response of human colonic epithelial cells to the adverse effects of the luminal compound sulfide. *Biochimica et biophysica acta*. 2005; 1725:201–212. [PubMed: 15996823]
- Olson KR. Hydrogen sulfide and oxygen sensing: implications in cardiorespiratory control. *J Exp Biol*. 2008; 211:2727–2734. [PubMed: 18723529]
- Olson KR. Hydrogen sulfide is an oxygen sensor in the carotid body. *Respiratory physiology & neurobiology*. 2011; 179:103–110. [PubMed: 21968289]
- Peers C, Wyatt CN, Evans AM. Mechanisms for acute oxygen sensing in the carotid body. *Respir Physiol Neurobiol*. 2010; 174:292–298. [PubMed: 20736087]
- Peng YJ, Nanduri J, Raghuraman G, Souvannakitti D, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. H₂S mediates O₂ sensing in the carotid body. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:10719–10724. [PubMed: 20556885]
- Prabhakar NR, Kumar GK, Chang CH, Agani FH, Haxhiu MA. Nitric oxide in the sensory function of the carotid body. *Brain Research*. 1993; 625:16–22. [PubMed: 7694772]
- Smith RP, Gosselin RE. Hydrogen sulfide poisoning. *J Occup Med*. 1979; 21:93–97. [PubMed: 556262]

- Van de Louw A, Haouzi P. Inhibitory effects of hyperoxia and methemoglobinemia on H(2)S induced ventilatory stimulation in the rat. *Respiratory physiology & neurobiology*. 2012; 181:326–334. [PubMed: 22490362]
- Wang ZZ, Stensaas LJ, Bredt DS, Dinger B, Fidone SJ. Localization and actions of nitric oxide in the cat carotid body. *Neuroscience*. 1994; 60:275–286. [PubMed: 7519759]
- Winder CV, Winder HO. The seat of action of sulfide on pulmonary ventilation. *Am J Physiol*. 1933; 105:337–352.
- Zapata, P.; Larraín, C.; Reyes, EP.; Fernández, R. Shifting from hypoxia to hyperoxia to assess the peripheral chemosensory drive of ventilation. In: CAN, et al., editors. *Arterial Chemoreception: From Molecules to Systems*. Springer Science; Dordrecht: 2012.

Highlights

- Acute hyperoxic exposure does not reduce H₂S-induced hyperpnea
- Acute hyperoxia not decrease the level of *dissolved* H₂S in the arterial blood during H₂S infusion.
- O₂ does not appear sensed in the carotid body through rapid and reversible alteration in H₂S rate of oxidation.

Figure 1.

Ventilatory response to 100% O₂ exposure in the same animal in control condition, during H₂S infusion, and H₂S infusion with 3% CO₂. Inspiratory flow ($\dot{V}$), minute ventilation ($\dot{V}_I$), and alveolar fraction of H₂S are displayed. Minute ventilation was stimulated during H₂S infusion; the depressive effect of pure O₂ exposure was the same in all 3 conditions, as a consequence, the stimulatory effect of H₂S was not affected by hyperoxia and ventilation during hyperoxia was higher during sulfide infusion than in control condition. Note that for clarity, hyperoxic tests with 3% CO₂, are depicted as 100% O₂ (rather than 97%)

Figure 2.

A) Averaged minute ventilation before and during 100% O₂ exposure (nadir response) in control condition, during H₂S infusion, and H₂S infusion with a background of 3% CO₂. In all conditions, minute ventilation decreased during 100% O₂ exposure; the level of minute ventilation was significantly higher during sulfide infusion than in the control tests. **B)** Difference between minute ventilation during hyperoxia ($V(H)_I$) and just before the Dejours test ($V(pre)_I$). Hyperoxic exposure decreased breathing in all conditions with a significantly higher depression during H₂S infusion in hypocapnic condition, however a large portion of the H₂S induced breathing stimulation was still preserved as illustrated in **C)** where the difference between minute ventilation during hyperoxia ($V(H)_I$) and baseline, i.e. prior to H₂S infusion, ($V(base)_I$) is illustrated. Note that when PaCO₂ was increased by few Torr during H₂S infusion, the depression of breathing during the Dejours test, was not different from control conditions. **D)** Level of arterial PCO₂ before and during hyperoxic exposure in control condition, H₂S infusion, and H₂S with 3% CO₂. PaCO₂ during H₂S, both in air and O₂ breathing, was lower than control conditions. **E)** Concentrations of gaseous form of H₂S before and during hyperoxia. Note the lack of decrease in CgH₂S during pure O₂ breathing. All data are presented as mean $\pm$ SD. [†] significantly different from corresponding pre-hyperoxic conditions, P<0.01. * significantly different from each corresponding control group (without H₂S infusion), P<0.01. # significantly different from H₂S without CO₂, P<0.01. Note that, like in Figure 1, hyperoxic tests with 3% CO₂ are depicted as 100% O₂ (rather than 97%).

Figure 3.

Example of the ventilatory response to H_2S infusion (7 $\mu\text{mol}/\text{min}$) during air breathing (left panel) and during the inhalation of 10% O_2 without sulfide infusion (right panel) in one rat. Inspiratory flow ($\dot{V}$), minute ventilation ($\dot{V}_I$), and alveolar fraction of H_2S are displayed. Minute ventilation was stimulated and the number of augmented breaths (arrow) was increased during both H_2S infusion and hypoxia. Note that the ventilatory responses to these 2 stimuli bear a striking resemblance.

Table 1

Summary of the averaged values of minute ventilation before and at the nadir of hyperoxic exposure along with the averaged PaCO₂ reached during the phase of hypoventilation induced by O₂

	Control (Air)	CO ₂	H ₂ S	H ₂ S + CO ₂
Baseline minute ventilation prior to O ₂ test (ml/min)	301 ± 51	338 ± 61 ^{\$}	379 ± 48 ^{\$}	411 ± 56 ^{\$}
Nadir of the response to hyperoxia (ml/min)	210 ± 43 [*]	266 ± 39 ^{*\$&}	254 ± 44 ^{*\$&}	308 ± 50 ^{*\$&}
PaCO ₂ during O ₂ exposure (Torr)	46 ± 3	47 ± 2.9	38 ± 6 ^{&}	41 ± 7

* significantly different from baseline minute ventilation for each condition,

^{\$} significantly different from baseline air breathing,

[&] significantly different from the hyperoxic control condition.

Note that hyperoxia dropped minute ventilation in all conditions but that the level of ventilation reached during hyperoxia with H₂S was higher than in control test and that hyperoxia with H₂S and CO₂ was not different from baseline air breathing. Also note that PaCO₂ was not different during O₂ only and CO₂+ O₂ as the level of ventilatory depression was more severe in the former (see method section for more details).