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Cardiac responses to 24hrs hyperoxia in *Bmp2* and *Bmp4* heterozygous mice

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Abstract

Background: Hyperoxia or clinical oxygen (O₂) therapy is known to result in increased oxidative burden. Therefore, understanding susceptibility to hyperoxia exposure is clinically important. Bone morphogenetic proteins (BMPs) 2 and 4 are involved in cardiac development and may influence responses to hyperoxia.

Methods: *Bmp2*^{+/-}, *Bmp4*^{+/-} and wild-type mice were exposed to hyperoxia (100% O₂) for 24 hrs. Electrocardiograms (ECG) were recorded before and during exposure by radio-telemetry.

Results: At baseline, a significantly higher low frequency (LF) and total power (TP) heart rate variability (HRV) were found in *Bmp2*^{+/-} mice only ($p < 0.05$). Twenty-four hours hyperoxia-induced strain-independent reductions in heart rate, QTcB and ST-interval and increases in QRS, LF HRV and standard deviation of RR-intervals were observed. In *Bmp4*^{+/-} mice only, increased PR-interval (PR-I) (24hrs), P-wave duration (P-d; 18 and 21–24hrs), PR-I minus P-d (PR-Pd; 24 hrs) and root of the mean squared differences of successive RR-intervals (24 hrs) were found during hyperoxia ($p < 0.05$).

Discussion: Elevated baseline LF and TP HRV in *Bmp2*^{+/-} mice suggests an altered autonomic nervous system regulation of cardiac function in these mice. However, this was not related to strain specific differences in responses to 24 hrs hyperoxia. During hyperoxia, *Bmp4*^{+/-} mice were the

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Declaration of interest

The authors report no declarations of interest.

most susceptible in terms of atrioventricular conduction changes and risk of atrial fibrillation, which may have important implications for patients treated with O₂ who also harbor *Bmp4* mutations. This study demonstrates significant ECG and HRV responses to 24 hrs hyperoxia in mice, which highlights the need to further work on the genetic mechanisms associated with cardiac susceptibility to hyperoxia.

Keywords

Bone morphogenetic protein; electrophysiology; oxidative stress

Introduction

High levels of reactive oxygen species (ROS) are well known to cause changes and/or damage to cellular structure and function. Oxygen (O₂) therapy is a major source of ROS and is commonly used in intensive care, for example, following post-myocardial resuscitation. Moreover, administration of 100% O₂ during initial resuscitation and 40–60% inspired O₂ in cases of acute breathlessness and emergency room (ER) assessment stage are currently recommend (Biarent et al., 2010; Murphy et al., 2001). The primary clinical goal is to deliver sufficient inspired O₂ concentration to achieve a O₂ saturation (SpO₂) of between 90 and 98% (Biarent et al., 2010; Murphy et al., 2001) with little attention given to the potentially harmful effects of elevated inspired O₂ content. Early adverse cardiovascular responses to elevated inspired O₂ (first 24 hrs; e.g. changes in heart rate (HR) and/or blood pressure; Kilgannon et al., 2010) may be predictive of poor prognoses. Management of hypoxemia in critically ill patients with O₂ therapy remains popular among clinicians but presents challenges in avoiding well-established deleterious effects, especially in the lung (Martin & Grocott, 2013). Moreover, post-cardiac arrest patients who develop high partial pressure of O₂ in arterial blood in the first 24hrs of intensive care hyperoxia had the lowest survival rates I compared to hypoxic or normoxic patients (Kilgannon et al., 2010). In the cardiovascular system, short-termSI hyperoxia has been reported to reduce coronary blood flow (McNulty et al., 2005) and myocardial O₂ uptake (Ganz et al., 1972). Despite these outcomes, which could be harmful to the patient, O₂ therapy is used in approximately 800 000 patients per year (Kim et al., 2008) and its mismanagement has been suggested to result in treatment errors that exceed the use of antibiotics (Boyle & Wong, 2006; Brokalaki et al., 2004; Hickey, 2007). This highlights the urgency for understanding the mechanisms for susceptibility to hyperoxia.

Recently, we reported significant reductions in HR during prolonged hyperoxia in C3H/HeJ and C57BL/6J mice (Howden et al., 2012) and sensitivity to these adverse responses concurred with strain-specific susceptibility to hyperoxia-induced lung injury (Hudak et al., 1993). However, the genetics of cardiac responses to short-term hyperoxia exposure are not well defined, but could help identify patients susceptible to O₂ therapy before organ dysfunction/damage occurs in response.

Genetic factors that may influence cardiac responses to hyperoxia include bone morphogenetic proteins (BMPs), which are members of the transforming growth factor (TGF)-p superfamily. BMP 2 and 4 have been associated with impaired cardiac development

in knockout mice. For example, *Bmp4* mutant murine embryos have been reported to suffer developmental defects in the atrioventricular (AV) septum (Jiao et al., 2003) and outflow tract septation (Liu et al., 1996). Moreover, BMP2 has been cited as critical for embryonic cardiac development (Ma et al., 2005; Zhang & Bradley, 1996).

Although null *Bmp2* and *Bmp4* embryos are non-viable (Dunn et al., 1997; Winnier et al., 1995; Zhang & Bradley, 1996), heart defects have also been reported in viable compound heterozygotes for these genes, including defects in the interventricular septum (Uchimura et al., 2009), which could affect QT-interval (QT-I) and PR-interval (PR-I). BMP signaling has also been associated with myocardial conduction pathways and AV node function (Stroud et al., 2007), providing further evidence for possible influences on QT-I and PR-I.

The clear importance of BMP2 and BMP4 signaling in cardiac development and function suggests these genes may be critical components to cardiac responses under conditions of oxidative stress. For example, *Bmp4* RNA levels have been shown to increase in mouse lung during hyperoxia-induced oxidative stress (Pogach et al., 2007). Furthermore, increased BMP4 levels induced oxidative stress, leading to endothelial dysfunction (Wong et al., 2010). In response to ischemia-reperfusion, known to induce oxidative stress, BMP4 promoted cellular apoptosis in the myocardium, which was attenuated in *Bmp4*^{+/-} mice, indicated by a reduced infarct size (Pachori et al., 2010). *Bmp2* expression increased significantly in coronary artery endothelial cells in response to hydrogen peroxide-induced oxidative stress (Csizsar et al., 2009; Dalfino et al., 2010). Treatment with BMP2 in post-myocardial infarction mice reduced oxidative stress burden and infarction size (Ebelt et al., 2013). This indicates that BMP2 and BMP4 might play important roles in cardiopulmonary responses to oxidative stress, and ergo a reduction in these proteins may affect responses to hyperoxia.

We therefore hypothesized that these pathways play a critical role in postnatal cardiac function, especially during environmental stress like hyperoxia since cardiac pathologies do not always present under resting conditions (e.g. exercise stress testing for suspected myocardial ischemia). In this study, we investigated HR, AV conduction timing and heart rate variability (HRV) in *Bmp4*^{+/-} or *Bmp2*^{+/-} mice both at baseline and in response to 24 hrs hyperoxia exposure.

Materials and methods

Animals

Bmp4 (*n* = 3) or *Bmp2* (*n* = 6) heterozygote mice, in addition to wild-type (WT) littermates (*n* = 6) were 12–24 weeks of age with a body mass range of 26.6–32.2 g. The *Bmp2* mice were generated by a targeted deletion of exon 2 in embryonic stem cells (Zhang & Bradley, 1996). The *Bmp4* heterozygous mice were generated by a targeted disruption of the first coding exon in the *Bmp4* gene (Winnier et al., 1995). In both lines, respective *Bmp2* and *Bmp4* expression levels have been shown to be significantly reduced by ~40% (Anderson et al., 2010; Cain & Bertram, 2006). Animals were housed individually with a 12:12 hr light-dark cycle. Food (NIH-31) and water were provided *ad libitum*. Animals were handled in accordance with The National Institutes of Health Humane Care and Use of Laboratory

Animals guidelines, following approval of procedures by the Animal Care and Use Committee.

Surgical implantation of radio-telemetry transmitter

Prior to survival surgery, mice were anesthetized with inhaled isoflurane, and buprenorphin (0.1mg/Kg) was given for analgesia. A 3 cm midline dorsal cutaneous incision was made, and a blunt instrument was used to create a subcutaneous tissue pocket. An ETA-F20 electrocardiogram (ECG) transmitter (DSI; Arden Hills, MN) was placed inside the tissue pocket, and positive and negative ECG leads were sutured over the left superficial gluteus and right trapezius muscles, respectively. All incisions were closed using wound clips, and animals were allowed five days to recover.

Measurements

Conscious, unrestrained animals were placed individually in whole body plethysmographs (Buxco Electronics, Wilmington, NC), which were used as exposure chambers and allowed at least 30 minutes to become quiescent before recording 20 minutes of baseline ECG (Ponemah, v4.80-SP4, St. Paul, MN). Recordings began at the same time of day from all mice to account for circadian variation. Food (NIH-31) and water were provided *ad libitum* during exposures.

Hyperoxia exposure

Following baseline data recording, mice were exposed to hyperoxia (100% O₂) for 24hrs in the whole body plethysmographs. O₂ was delivered from a liquid O₂ tank, warmed to room temperature and humidified by passing the gas through a sealed water-containing flask. O₂ was delivered to the each plethysmograph at 1 liter per minute to match the Buxco bias-flow pump flow rates. Inside the plethysmographs, mice were placed on a standard Buxco platform within the chamber, which has holes to allow urine and fecal matter to fall through to the bottom of the chamber. This arrangement ensured that mice were not exposed to urine or fecal matter during exposures.

Data and statistical analysis

HR, QT-I (beginning of the Q-wave to the end of the T-wave; corrected using Bazett's formula; QTcB), QRS-interval (QRS;beginning of Q-wave to the end of the S-wave), ST-interval (ST-I; end of S-wave to the end of the T-wave), PR-I (beginning of the P-wave to the beginning of the S-wave) and P-wave duration (P-d; beginning to the end of the P-wave) data were calculated using ECG pattern recognition software (Ponemah, v4.8-SP4). AV node conduction delay was assessed by subtracting P-d from PR-I.

The standard deviation of RR-intervals (SDNN) and the square root of the mean squared differences of successive RR-intervals (RMSSD) HRV measures were calculated using standard practices (Task Force, 1996). Frequency domain HRV was calculated using Ponemah, v4.8 SP-4. As described previously (Howden et al., 2008), using 2-minute samples, the frequency ranges used were 02–1.5 Hz (low frequency; LF) and 1.5–5.0Hz (high frequency; HF). Total power (TP) was determined by the summation of corresponding LF and HF HRV values.

Between-strain differences in baseline ECG and HRV phenotypes were assessed separately using one-way analyses of variance (ANOVAs) and Holm-Sidak post-hoc analyses when appropriate. To assess phenotypic responses to 24hrs hyperoxia, we used repeated measures ANOVAs to test for the effect of strains, time and the strains x time interaction on each of the 12 traits. In this model, the individual mice were the subjects (a random factor) with time as the within-subject (repeated) factor and strain as the between-subject factor. A significant strain effect denoted differences among the three strains (over all pooled time periods), and where this occurred, we used Tukey's *post-hoc* tests to test for each of the three individual pairwise comparisons among the three strains. A significant time effect for any trait suggested differences among the 24 hr time periods (over all pooled strains), and where this occurred, we used Dunnett's *post-hoc* tests to test for the difference between the trait after one hour of hypoxia versus all other times. A significant strain by time interaction suggested that strain effects varied over the time periods, and where this occurred, we did not evaluate the main effects of either strain or time and instead used Tukey's *post-hoc* tests to test individual strain differences at each of the time periods. It should be emphasized that these *post-hoc* tests are based on error variances calculated across the entire sample, and therefore the magnitude of individual standard errors for any two means are not reliable indicators of potential significance between these means. Strain x time comparisons were made to 1 hr hyperoxia and not baseline because mice did not always remain quiescent when hyperoxia began. The repeated-measures ANOVA and *post-hoc* calculations were done using SAS software, version 9.2 (Cary, NC) (SAS Institute Inc., 1997).

Results

Baseline measures versus strain

There were no significant differences in baseline HR (616.5 ± 34.0 *Bmp4*^{+/-}, 612.1 ± 32.9 *Bmp2*^{+/-} and 578.1 ± 20.7 WT bpm; Figure 1), QTcB, QRS, ST-I (236.2 ± 5.4 *Bmp4*^{+/-}, 230.3 ± 9.1 *Bmp2*^{+/-}, 222.1 ± 13.7 WT ms; 13.5 ± 0.6 *Bmp4*^{+/-}, 14.3 ± 0.2 *Bmp2*^{+/-}, 14.1 ± 0.3 WT ms; 61.6 ± 3.3 *Bmp4*^{+/-}, 56.5 ± 4.6 *Bmp2*^{+/-}, 66.2 ± 3.6 WT ms, respectively; Figure 2), PR-I, P-d and PR-I minus P-d (25.2 ± 1.2 *Bmp4*^{+/-}, 26.2 ± 1.5 *Bmp2*^{+/-}, 24.7 ± 1.4 WT; 12.8 ± 0.2 *Bmp4*^{+/-}, 12.8 ± 0.2 *Bmp2*^{+/-}, 12.8 ± 0.2 WT; 12.8 ± 0.2 *Bmp4*^{+/-}, 13.3 ± 0.3 *Bmp2*^{+/-}, 13.2 ± 0.8 WT; 12.3 ± 1.0 *Bmp4*^{+/-}, 12.8 ± 1.3 *Bmp2*^{+/-}, 12.1 ± 1.2 WT, respectively; Figure 3) between the three strains.

No between-strain differences were found in SDNN (12.2 ± 3.3 *Bmp4*^{+/-}, 12.8 ± 1.7 *Bmp2*^{+/-} and 9.2 ± 1.7 WT ms; Figure 4) or RMSSD HRV at baseline. However, in *Bmp2*^{+/-} mice, significantly higher LF HRV (0.15 ± 0.02 ; $p = 0.04$) was found compared to *Bmp4*^{+/-} (0.06 ± 0.006) and WT (0.07 ± 0.004) mice, but HF HRV was not different between strains (0.05 ± 0.03 *Bmp4*^{+/-}, 0.09 ± 0.02 *Bmp2*^{+/-}, 0.05 ± 0.02 WT). Because TP HRV is the summation of LF and HF HRV, a significantly higher TP HRV was found in *Bmp2*^{+/-} mice (0.21 ± 0.03 ; $p = 0.02$) compared with *Bmp4*^{+/-} (0.11 ± 0.03) and WT (0.12 ± 0.02) mice.

Responses to hyperoxia at all time points versus strain

Twenty-four hours hyperoxia exposure resulted in a significant reduction in HR when data from all strains were pooled (578.1 ± 15.7 bpm at 1 hr versus 436.0 ± 43.3 bpm at 24hrs; $p < 0.01$; Table 1; Figure 1). Significant HR reductions compared to 1 hr hyperoxia were identified at 18–24 hrs exposure ($p < 0.05$; Figure 1; Table 2). However, there was no time $\times$ strain effect.

Twenty-four hours hyperoxia also induced a significant reduction in QTcB when all strain data were pooled (220.8 ± 8.1 ms at 1 hr versus 181.6 ± 12.5 ms at 24hrs; $p < 0.01$; Table 1; Figure 2), but no time $\times$ strain effect was found. QRS interval increased (13.8 ± 0.2 ms at 1hr versus 14.5 ± 0.3 ms at 24 hrs; $p < 0.01$; Table 1; Figure 2) and ST-I decreased (61.4 ± 1.5 ms at 1hr versus 51.4 ± 2.6 ms at 24hrs; $p < 0.01$; Table 1; Figure 2) significantly in response to 24 hrs hyperoxia exposure. No between-strain differences were found for these phenotypes.

During 24 hrs hyperoxia, there were significant increases in pooled PR-I (27.5 ± 0.7 ms at 1 hr versus 36.1 ± 2.1 ms at 24 hrs; $p < 0.01$; Table 1; Figure 3), in addition to a time $\times$ strain effect ($p < 0.01$; Table 1). *Post-hoc* analysis revealed a significantly higher PR-I in *Bmp4*^{+/-} mice at 24 hrs hyperoxia compared *Bmp2*^{+/-} mice (44.7 ± 9.3 ms *Bmp4*^{+/-} versus 34.7 ± 5.5 ms *Bmp2*^{+/-}; $p < 0.01$; Figure 3).

When assessing P-d and PR-Pd dynamics during hyper-oxia, changes in PR-I were accounted for primarily by changes in P-d. Analysis of pooled P-d and PR-Pd showed a significant increase in both phenotypes during 24hrs hyperoxia (P-d 13.4 ± 0.2 ms at 1 hr versus 18.7 ± 1.8 ms at 24hrs; PR-Pd 14.1 ± 0.8 ms at 1 hr versus 18.7 ± 1.7 ms at 24hrs $p < 0.01$; Table 1; Figure 3). Moreover, there was a time $\times$ strain interaction for both P-d ($p < 0.01$) and PR-Pd ($p < 0.01$). However, post-hoc analysis did not identify any specific time points at which strains differences between PR-Pd were evident.

Twenty-four hours hyperoxia induced increases in both time-domain measures of HRV. Significant increases in pooled SDNN (10.7 ± 0.9 ms at 1 hr versus 20.8 ± 4.5 ms at 24hrs) and RMSSD (5.8 ± 0.5 ms at 1 hr versus 8.7 ± 2.7 ms at 24hrs) were found. Post-hoc analysis of SDNN identified significant increases from 21 to 24hrs hyperoxia ($p < 0.05$; Table 2; Figure 4). Furthermore, a strain $\times$ time interaction was identified in RMSSD during hyperoxia ($p < 0.01$; Table 1). However, post-hoc analysis did not identify specific time periods during hyperoxia when differences were strain dependent.

An effect of 24 hrs hyperoxia on frequency domain HRV was observed in the LF HRV range. Pooled LF HRV increased during hyperoxia a small, but significant amount (0.09 ± 0.01 ms at 1hr versus 0.11 ± 0.01 ms at 24hrs; $p < 0.01$; Table 1; Figure 5). No changes were found in HR HRV or TP HRV ($p > 0.05$; Table 1; Figure 5). In addition to time main effects in LF HRV, differences were found between strains when all strain specific hyperoxia data were pooled in LF, HF and TP HRV (Table 3), but these strain differences did not influence responses to hyperoxia as no time $\times$ strain interaction was found.

Responses to hyperoxia at 1, 6, 12, 18 and 24hrs hyperoxia versus strain

Because our statistical analysis using all time points during 24 hrs hyperoxia assessing time x strain interactions resulted in 48 degrees of freedom, statistical power to detect differences between strains was significantly reduced. Therefore, we further analyzed our data comparing only 1, 6, 12, 18 and 24hrs hyperoxia. This statistical design identified additional differences between strains that were of interest. Strain differences were identified at 24 hrs hyperoxia for PR-I, P-d, PR-Pd and RMSSD ($p < 0.05$; Figures 3 and 4).

Discussion

Baseline measures versus strain

BMP2 and BMP4 have been implicated in cardiac development and function (Jiao et al., 2003; Stroud et al., 2007; Uchimura et al., 2009; van Wijk et al., 2007), and so disruption of *Bmp2* or *Bmp4* could lead to functional abnormalities in adulthood. However, in mice heterozygous for *Bmp2* or *Bmp4*, no significant differences were found in baseline HR QTcB or PR-I (or their components; Figures 1–3), which were within previously reported normal phenotypic ranges (Howden et al., 2008; Wehrens et al., 2000; Xing et al., 2009). Moreover, no differences between strains were found in baseline SDNN or RMSSD (Figure 4). It is possible that in mice heterozygous for *Bmp2* or *Bmp4*, cardiac development was not limited to the extent that functional differences were detectable by ECG or the effect did not impact cardiac function when mice were quiescent.

However, baseline LF HRV was significantly higher in *Bmp2*^{+/-} mice compared the *Bmp4*^{+/-} and WT mice (Figure 5), which was reflected in the pattern of strain differences in TP HRV. Interpreting changes LF HR oscillations is challenging and still a matter of contention in the literature (Malpas, 2002). While it is common to cite changes in LF HRV as alternations in sympathetic outflow oscillations, this seems to be an inadequate explanation especially considering the lack of a difference in baseline HR between the strains used in this study. LF HRV is more likely under the influence of blood pressure control mechanisms (Reyes Del Paso et al., 2013) or multiple cardiovascular control mechanisms, including baroreceptor feedback (Van Roon et al., 2004). Regardless, these data do suggest that reduced *Bmp2* expression influences cardiovascular regulation in adult mice, although the consequences of this difference are not clear in these data.

Responses to hyperoxia versus strain

Significant hyperoxia-induced cardiac responses were found in all phenotypes, except HF and TP HRV (Figures 1–5). Although this influence of hyperoxia was observed when data from all three strains was pooled, it demonstrates an important effect of short-term (24 hrs) hyperoxia exposure on several components of cardiac function. Prolonged exposure (3 days) of rodents to hyperoxia is lethal (Clerch & Massaro, 1993), and we have reported a link between the deleterious pulmonary effects of hyperoxia and severe bradycardia (Howden et al., 2012). Interestingly, hyperoxia-induced pulmonary edema in mice does not occur until 48 hrs hyperoxia, even in susceptible C57BL/6J mice (Howden et al., 2012; Hudak et al., 1993). It is important to note that these mice were young and healthy and therefore may have been better able to tolerate the influence of hyperoxia on cardiac

function. Therefore, the observed responses may have been more serious if the mice were aged or had pre-existing cardiopulmonary disease. This suggests that short-term hyperoxia exposure may have important clinical implications during the common practice of O₂ therapy (Mach et al., 2011).

Evidence for increases in pulmonary oxidative stress and inflammation (increased 8-isoprostane and Interleukin-6, respectively, in exhaled condensate) after only 1hr of 28% O₂ in healthy human subjects has been reported (Carpagnano et al., 2004). Moreover, 10, 30 or 90 minutes of 100% O₂ in rats all lead to an increase in oxidative damage and infiltration of inflammatory cells in post-exposure lung lavage supernatant (Valencia Sdos et al., 2007). These data demonstrate that short-term hyperoxia, even when O₂ content is only slightly increased (Carpagnano et al., 2004), are possible and therefore pulmonary alterations may have played an important role in the cardiac responses observed in this study.

Twenty-four hours hyperoxia induced a significant reduction in HR, QTcB, QRS duration and ST-I (Figures 1 and 2). One-hour hyperoxia exposure has been reported to result in reduced HR, stroke index and cardiac index (Lund et al., 1999; Thompson et al., 2006; Waring et al., 2003). These effects of short-term hyperoxia may be associated with a hyperoxia-induced reduction in myocardial O₂ uptake (Ganz et al., 1972), possibly in relation to increased coronary vascular resistance and reduced coronary blood flow (McNulty et al., 2005). However, precisely how these responses to acute hyperoxia relate to the responses observed in this study over a 24 hr period is not clear. For example, from Figure 1, it appears that at least 12 hrs hyperoxia is required before noteworthy changes in HR occur.

Short QT-I syndrome (SQTS) has been associated with increased risk of atrial fibrillation (AF) (Gussak et al., 2000) and an over twofold increase in sudden death risk (Algra et al., 1993). Interestingly, criteria for SQTS is not well defined in humans or rodents and human diagnosis requires a familial component (Hedley et al., 2009), suggesting genetics are important, although disruption to *Bmp2* or *Bmp4* did not affect responses to 24 hrs hyperoxia in this study. Since treatment of hypoxemic patients with hyperoxia is common in a wide-range of clinical circumstances, this study demonstrates a risk of developing SQTS during O₂ treatment that could have important clinical implications, including increased risk of developing atrial arrhythmia. The relative contributions of QRS and ST-I to QTcB responses were not evident from these data, but may have contributed equally.

Twenty-four hours hyperoxia exposure also induced significant increases in PR-I in the *Bmp4*^{+/-} mice compared to *Bmp2*^{+/-} and WT mice (Figure 3) providing further evidence for the importance of atrial function in this model. This implies an effect of hyperoxia on the AV conduction system of *Bmp4*^{+/-} mice, which may be associated with greater risk of deleterious responses to hyperoxia. Interestingly, increases in P-d were evident at 21 hrs hyperoxia in the *Bmp4*^{+/-} mice compared to *Bmp2*^{+/-} mice, but no changes in PR-Pd, or AV node impulse delay, were found until 24 hrs hyperoxia exposure. These results indicate that in *Bmp4*^{+/-} mice, but not in *Bmp2*^{+/-} mice, 24 hrs hyperoxia was associated with intra-atrial conduction delay, which has been reported to increase the risk of atrial flutter or AF (Buxton et al., 1984; Cosio et al., 1983). Previously, hyperoxic breathing has been reported to

prolong AV conduction time and reduced ventricular reactivity to AF (Daly et al., 1963). Furthermore, abnormal AV conduction has been reported with targeted deletion of the *Bmpr1a* receptor (Stroud et al., 2007) for which BMP4 is a ligand. These data point to a link between hyperoxia exposure and increased risk of atrial conduction disturbances when *Bmp4* is disrupted. However, it should be noted that BMP2 is also a ligand for the *Bmpr1a* receptor, suggesting either a differential influence of these two proteins on *Bmpr1a* receptor regulation of atrial function, or that this receptor was not important in the atrial conduction responses observed in this study.

Assessment of autonomic nervous system regulation of HR by time and frequency domain analysis of RR interval series has become well established, despite continued discussion about the origins of changes in these oscillations (Malpas, 2002; Reyes Del Paso et al., 2013). Twenty-four hours hyperoxia did not induce strain-specific responses in SDNN or frequency domain HRV, despite a higher baseline LF and TP HRV in *Bmp2*^{+/-} mice that was persistent throughout exposure (Figure 5). However, RMSSD did increase in *Bmp4*^{+/-} mice relative to *Bmp2*^{+/-} and WT mice at 24 hrs hyperoxia (Figure 4). RMSSD has been reported as an index for cardiac vagal modulation (Buchheit et al., 2007; Goldberger et al., 2006). This may be consistent with these data since the significant increase in RMSSD could have been associated with a greater trend for reduced HRs in *Bmp4*^{+/-} mice (Figure 1). However, the timing of the changes in these phenotypes suggests a degree of independence, assuming equal sensitivity of each measure. Moreover, suggesting an association between changes in RMSSD and HR is speculative since strain-specific differences in HR responses to hyperoxia were not found.

Nevertheless, oxidative stress and pro-inflammatory mediator release in the lung have been associated with vagal afferent nerve stimulation (Undem & Carr, 2001; Widdicombe & Lee, 2001). Since pulmonary oxidative stress and pro-inflammatory mediator release has already been established during short-term hyperoxia exposure, it is possible that pulmonary responses to hyperoxia contributed to the atrial conduction prolongation and increased RMSSD observed in this study. However, this does not necessarily account for the differences in PR-I, P-d, PR-Pd and RMSSD hyperoxia response between WT, *Bmp2*^{+/-} and *Bmp4*^{+/-} mice. BMP2 and BMP4 have been implicated in producing oxidative stress *via* NAD(P)H oxidase activation (Csiszar et al., 2006; Sorescu et al., 2004), suggesting reduced *Bmp2* or *Bmp4* expression would be protective against hyperoxia-induced oxidative stress. Since we did not observe any effect on AV conduction or RMSSD in *Bmp2*^{+/-} mice during hyperoxia, but only in *Bmp4*^{+/-} mice, possibly via vagal stimulation (Sapire et al., 1979), this suggests a differential effect of changes in *Bmp2* versus *Bmp4* expression in response to hyperoxia.

Conclusion

In this study, we have shown that partial disruption of *Bmp2* or *Bmp4* did not affect HR, QT-I, PR-I (or their components) or time domain measures of HRV in quiescent mice. However, in *Bmp2*^{+/-} mice, a significantly higher LF and TP HRV was found suggesting altered regulation of cardiovascular function. During hyperoxia, we found significant changes in PR-I, P-d, PR-Pd and RMSSD in *Bmp4*^{+/-}, but not in the other strains, which may have been

associated with pulmonary responses. Hyperoxia-induced delays in atrial conduction in *Bmp4*^{+/-} mice, which have been associated with atrial dysfunction, suggest genotype-dependent susceptibility to hyperoxia, the mechanisms of which deserve further study. Interestingly, none of the phenotypes assessed in this study changed in *Bmp2*^{+/-} mice specifically during hyper-oxia, suggesting that reduced expression of *Bmp2* does not influence responses to 24 hrs hyperoxia. This is a noteworthy contrast despite such striking structural and biochemical similarities between BMP2 and BMP4. These genetic determinants of susceptibility to cardiac dysfunction during 24hrs hyperoxia could have important implications for health outcomes when hyperoxia is used to treat hypoxemic patients.

Relevance of the research to humans

This study is the first to show that disruption of *Bmp2* or *Bmp4* did not affect most cardiac function phenotypes assessed in this study during quiescence, except for LF and TP HRV. However, these genotypes were associated with differential responses 24 hrs hyperoxia, especially in PR-I and its related components *Bmp4*^{+/-} mice. Short-term (24hrs) exposure of hypoxemic patients to hyperoxia is a common clinical practice, but little is known about differential susceptibility to O₂ toxicity among patients. This study suggests primarily that patients harboring mutations in *Bmp4* may be susceptible to atrial conduction dysfunction during O₂ therapy (hyperoxia).

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Bmp2^{+/-} and Bmp4^{+/-} cardiac responses to hyperoxia

Figure 1.

HR at baseline (bars) and in response to hyperoxia (line and symbol). Dark gray symbols = WT, light gray symbols = *Bmp2^{+/-}* and black symbols = *Bmp4^{+/-}* mice. Group means $\pm$ SEM are presented.

Figure 2.

QTcB, QRS and ST-I at baseline (bars) and in response to hyperoxia (line and symbol). Dark gray symbols = WT, light gray symbols = *Bmp2*^{+/-} and black symbols = *Bmp4*^{+/-} mice. Group means $\pm$ SEM are presented.

Figure 3.

PR-I, P-d and PR-I minus P-d at baseline (bars) and in response to hyperoxia (line and symbol). Significant differences ($p < 0.05$) are indicated by * = different from $Bmp2^{+/-}$ mice. † = from $Bmp2^{+/-}$ mice ($p < 0.001$) and ‡ = different from WT mice ($p < 0.01$). Dark gray symbols = WT, light gray symbols = $Bmp2^{+/-}$ and black symbols = $Bmp4^{+/-}$ mice. Group means $\pm$ SEM are presented.

Figure 4.

Time domain HRV at baseline (bars) and in response to hyperoxia (line and symbol). † = from *Bmp2*^{+/-} mice ($p < 0.001$) and ‡ = different from WT mice ($p < 0.01$). Dark gray symbols = WT, light gray symbols = *Bmp2*^{+/-} and black symbols = *Bmp4*^{+/-} mice. Group mean $\pm$ SEM presented

Figure 5.

Frequency domain HRV at baseline (bars) and in response to hyperoxia (line and symbol). * = different from *Bmp2*^{+/-} and WT mice ($p < 0.05$). Dark gray symbols = WT, light gray symbols = *Bmp2*^{+/-} and black symbols = *Bmp4*^{+/-} mice. Group mean $\pm$ SEM presented.

Table 1.

Repeated measures ANOVA results.

	<i>F</i> values		
	Strain (S)	Time (T)	S × T
HR	1.58	12.82 ^a	0.84
QTcB	0.83	4.74 ^a	1.27
QRS	0.38	6.36 ^a	0.96
ST-I	0.20	7.02 ^a	1.01
PR-I	1.74	15.39 ^a	2.31 ^a
P-d	3.40	10.43 ^a	1.88 ^a
PR-I-Pd	0.54	10.23 ^a	1.82 ^a
SDNN	1.04	3.75 ^a	1.16
RMSSD	0.99	4.92 ^a	1.76 ^a
LF	4.90 ^b	2.76 ^a	1.09
HF	6.62 ^b	1.23	0.55
TP	5.89 ^b	0.49	0.59

Shown are the *F* values for strains (2 d.f.), time (24d.f.), and their interaction (48 d.f.) from the repeated measures analyses of variance for each of the twelve traits.

^a = $p < 0.01$;

^b = $p < 0.05$.

Table 2.

Post-hoc analysis results for pooled strain time effects of hyperoxia.

Phenotype	Hours of hyperoxia at initial significance	Initial significance time point trait value change from 1 hr hyperoxia	24 hr trait value change from 1 hr hyperoxia
Heart rate (bpm)	18	-134.6 ± 27.8^a	-177.6 ± 36.4^b
QTcB (ms)	17	-68.5 ± 25.8^a	-35.1 ± 25.4^a
QRS (ms)	17	0.5 ± 0.1^c	0.7 ± 0.2^b
ST-I (ms)	16	-8.3 ± 3.6^a	-10.8 ± 2.7^c
SDNN HRV	21	10.5 ± 3.9^c	10.4 ± 5.1^c

Shown are phenotypes that changed significantly during hyperoxia, time at which significance was first determined and phenotypic changes from 1hr hyperoxia.

^a
 $p < 0.05$

^b
 $p < 0.001$

^c
 $p < 0.01$.

Table 3.

Overall strain differences in response to 24hrs hyperoxia.

Phenotype	BMP2 versus BMP4	BMP2 versus BMPWT	BMP4 versus BMPWT
LF	0.12 ± 0.01 versus 0.07 ± 0.02 (<i>ns</i>)	0.12 ± 0.01 versus 0.07 ± 0.01 ^a	0.07 ± 0.02 versus 0.07 ± 0.01 (<i>ns</i>)
HF	0.08 ± 0.01 versus 0.04 ± 0.01 ^a	0.08 ± 0.01 versus 0.04 ± 0.01 ^a	0.04 ± 0.01 versus 0.04 ± 0.01 (<i>ns</i>)
TP	0.20 ± 0.02 versus 0.12 ± 0.02 (<i>ns</i>)	0.20 ± 0.02 versus 0.12 ± 0.01 ^a	0.12 ± 0.02 versus 0.12 ± 0.01 (<i>ns</i>)

Shown are strain differences determined when all hyperoxia time points were pooled.

^{ns} = p>0.05.^a = p<0.05