Transient Neonatal High Oxygen Exposure Leads to Early Adult Cardiac Dysfunction, Remodeling, and Activation of the Renin–Angiotensin System

Mariane Bertagnolli, Fanny Huyard, Anik Cloutier, Zackary Anstey, Julie-Émilie Huot-Marchand, Catherine Fallaha, Pierre Paradis, Ernesto L. Schiffrin, Denis deBlois, Anne Monique Nuyt

Abstract—Perinatal conditions (such as preterm birth) can affect adult health and disease, particularly the cardiovascular system. Transient neonatal high O₂ exposure in rat in adulthood (a model of preterm birth–related complications) leads to elevated blood pressure, vascular rigidity, and dysfunction with renin–angiotensin system activation. We postulate that neonatal hypertensive stress also affects myocardial structure, function, and expression of renin–angiotensin system components. Sprague-Dawley pups were kept with their mother in 80% O₂ or in room air (control) from days 3 to 10 of life. Left ventricular function was assessed in 4-, 7-, 12-week-old (echocardiography) and in 16-week-old (intraventricular catheterization) male O₂-exposed versus control rats. At 16 weeks, hearts from O₂-exposed rats showed cardiomyocyte hypertrophy, enhanced fibrosis, and increased expression of transforming growth factor-β1, senescence-associated proteins p53 and Rb, upregulation of angiotensin II type 1 (AT1) receptor expression (protein and AT1a/b mRNA), and downregulation of AT2 receptors. At 4 weeks (before blood pressure increase), the expression of cardiomyocyte surface area, fibrosis, p53, and AT1b was significantly increased and AT2 decreased in O₂-exposed animals. After 4 weeks of continuous angiotensin II infusion (starting at 12 weeks), O₂-exposed rats developed severe heart failure, with impaired myocardial mechanical properties compared with saline-infused rats. Transient neonatal O₂ exposure in rats leads to left ventricular hypertrophy, fibrosis and dysfunction, and increased susceptibility to heart failure under pressure overload. These results are relevant to the growing population of individuals born preterm who may be at higher risk of cardiac dysfunction when faced with increased peripheral resistance associated with hypertension, vascular diseases, and aging. (Hypertension. 2014;63:143-150.) • Online Data Supplement

Key Words: angiotensin II receptors ▪ endomyocardial fibrosis ▪ heart failure ▪ oxidative stress ▪ preterm birth ▪ senescence

Twelve percent of infants are born preterm (before 37 weeks of gestation) and 1.5% extremely preterm (before 29 weeks). Since the mid-1980s, ≥90% of these infants survive, including the most premature ones; accordingly, it is estimated that currently ≥10% of all young adults were born preterm and 1% very preterm (March of Dimes; http://www.marchofdimes.com/mission/globalpreterm.html). Long-term health consequences of preterm birth are only starting to be revealed, and recent studies of adolescents and young adults report higher blood pressure (BP), increased incidence of hypertension, and indices of vascular dysfunction; however, the impact of preterm birth on cardiac structure and function in adults is unknown.

Preterm infants have lower and less inducible antioxidant defences and are exposed on birth to high concentrations of O₂, relative to intrauterine life and to pro-oxidant conditions. This oxidative injury is an important pathogenic factor in classical short-term complications of prematurity (such as retinopathy of prematurity and bronchopulmonary dysplasia). Experimentally, we have shown that neonatal high O₂ exposure in young adult rats leads to elevated BP, vascular dysfunction with increased response to angiotensin II (Ang II) suggesting renin–angiotensin system (RAS) activation, and increased vascular stiffness. However, the impact of high O₂ exposure on heart development and cardiac function remains unknown. Cardiomyocytes are in active proliferation ≤36 weeks of gestational age in humans and ≤2 weeks after birth in rats. We therefore postulate that, in susceptible/immature individuals, deleterious neonatal conditions such as exposure

DOI: 10.1161/HYPERTENSIONAHA.113.01760
to high O2 can significantly affect the development of myocardial structure and function. Further, affected individuals could be at higher risk of cardiac dysfunction when faced with increased peripheral resistance and BP elevation.

In cardiovascular disease, RAS plays a major role in vascular dysfunction, cardiac hypertrophy, and fibrosis through Ang II acting predominantly on its type I receptor (AT1)10,11 triggering downstream mechanisms such as the profibrotics transforming growth factor-β1 (TGF-β1) and hypoxic-inducible factor-1α (HIF-1α),12 as well as senescence-associated pathways.13 Activation of the RAS in the kidneys, brain, and systemic vasculature plays a key role in the elevation and maintenance of BP in experimental models associated with deleterious perinatal conditions including transient neonatal high O2 exposure.5,14

However, the effect of neonatal pro-oxidant conditions on cardiac RAS components has not been reported.

To determine whether neonatal hyperoxia exposure impaired cardiac development and predisposed to cardiac dysfunction, myocardial structure, and function, expression of cardiac RAS components, as well as TGF-β1, HIF-1α, and senescence-associated proteins, was determined in young, 4-week-old, and adult, 16-week-old rats infused or not with Ang II.

Materials and Methods

Sprague-Dawley pups were kept with their mother in 80% O2 using an oxycycler (ProOx P110, Biosherix, Lacona, NY) from days 3 to 10 of life (group O2, n=9 litters) as previously described.4 To avoid maternal morbidity associated with O2 toxicity, the mother was interchanged every 24 hours with a surrogate mother of a litter maintained at room air (NO group). Pups from the control group were maintained in room air with their mother (without interchange; n=9 litters). Pups were weaned at 4 weeks to regular chow. Only male offspring were studied. Results obtained from NO group are not different to controls, suggesting no deleterious effect of neonatal intermittent separation from mothers (data not shown). The number of pups per litter was culled to 12 (6 males and 6 females) and no >3 animals (males) per litter were used in the study, for a total n=27 rats per group. At 4, 7, and 12 weeks, systolic BP (SBP) and heart rate were assessed by the tail-cuff method (50-001 Rat Tail BP System, Cupertino, CA) implanted under inhaled isoflurane anesthesia (2:1 isoflurane:O2) with the Animal Ethics Committee of the Hospital Sainte-Justine Research Center and followed the guidelines of the Canadian Council on Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results

Cardiac Hypertrophy and Dysfunction in Young and Adult O2-Exposed Versus Control Rats

As previously shown,4 tail-cuff SBP was significantly increased at 7 and 12 weeks, but not at 4 weeks, and heart rate was significantly increased at 12 weeks, but not at 4 and 7 weeks, in O2-exposed versus control animals (Table). Body weight was not different between controls and O2 at all time points studied. At 4 weeks, reduced systolic function assessed by fractional shortening (FS) that is characterized by an increased LV internal diameter in systole was observed in O2-exposed versus controls. At 7 weeks, O2-exposed rats presented LV concentric hypertrophy, revealed by an increase in the LV posterior wall thickness in diastole and LV mass, a decrease in systolic and diastolic function indicated by smaller FS (characterized by an increased LV internal diameter in systole) and mitral valve peak velocity of the mitral E wave and in E/A ratio and increased deceleration time. At 12 weeks, the heart further remodelled to develop LV eccentric (or dilated) cardiac hypertrophy with systolic and diastolic dysfunction in O2-exposed rats. The intraventricular septal thickness in diastole, LV posterior wall thickness in diastole, LV internal diameter in diastole, and LV internal diameter in systole and LV mass were increased, whereas FS and mitral valve peak velocity of the mitral E wave and in E/A ratio were decreased and deceleration time was increased in O2-exposed versus controls rats.

Intra-arterial BP recordings in conscious animals were higher in the 16-week-old O2-exposed versus control animals (SBP: 151±3 versus 110±3; mean BP: 134±4 versus 93±2; diastolic BP: 125±5 versus 84±2 mm Hg, respectively; all P<0.001; n=6 per group). Heart rate was not different between groups (323±7 versus 344±15 bpm). LV end-diastolic pressure was increased by 6 mm Hg, and maximum (+dP/dT) and minimum (−dP/dT) derivatives reduced in O2-exposed rats (Figure 1A–1C), indicating impaired LV systolic and diastolic function versus control animals. The heart/body weight ratio, a cardiac hypertrophic index, was slightly increased in O2-exposed animals (2.82±0.03 versus 2.67±0.06 mg/g; P=0.03; n=6–8 per group), whereas body weight was not changed (567±13 O2-exposed versus 559±22 g) compared with control animals.

Histological analysis of LV cardiac sections at 4 (Figure 2A and 2B) and 16 weeks (Figure 2C and 2D) showed an increase in cardiomyocyte cross-sectional area in O2-exposed rats (Figure 2E). No difference in cardiomyocyte cell numbers was observed between groups at 4 (O2-exposed: 1.07±0.15 versus controls: 1.33±0.15×103 cells per LV; n=5 per group) and 16 weeks (O2-exposed: 1.52±0.12 versus controls: 1.74±0.14×103 cells per LV; n=5 per group). Myocardial interstitial fibrosis (Masson trichrome staining) was increased in O2-exposed rats at 4 and 16 weeks (Figure 3A and 3B). Expression of profibrotic factor TGF-β1 was similar between groups at 4 weeks but increased in O2-exposed at 16 weeks (Figure 3C). HIF-1α, however, was more expressed in O2-exposed at 4 and 16 weeks (Figure 3D). Senescence-associated proteins p53 and Rb were increased in the myocardium of O2-exposed 16-week-old rats;
Expression of the RAS Components

AT1 receptor protein expression and AT1a mRNA in the LV were not different between groups at 4 weeks but increased in O2-exposed animals at 16 weeks (Figure 5A and 5B). AT1b mRNA expression was increased in both young (4 weeks) and adult (16 weeks) O2-exposed groups (Figure 5B). AT2 protein and gene expressions were significantly reduced at 4 and 16 weeks in O2-exposed rats (Figure 5A and 5B). No difference between groups was observed in angiotensin-converting enzyme protein expression at either age studied (Figure 5A).

Impact of Ang II Infusion on Hemodynamic Parameters and Cardiac Function

SBP (measured in the carotid under isoflurane anesthesia; Figure 6A) and the heart/body weight ratio (Figure 6B) similarly increased after 2 weeks of Ang II infusion (initiated at 12 weeks) in control and O2-exposed groups; no further increase was observed after 4 weeks of infusion. In saline-infused animals, there was no difference in SBP between groups, but heart/body weight ratios were higher in O2-exposed versus control animals after 2 or 4 weeks of infusion. LV end-diastolic pressure in control animals increased after 2 weeks of Ang II infusion without further increase after 4 weeks, whereas in O2-exposed animals LV end-diastolic pressures that are elevated by guest on August 30, 2017 http://hyper.ahajournals.org/ Downloaded from at 4 weeks, only p53 was significantly increased and no difference between groups was noted for Rb (Figure 4A and 4B).

Table. Hemodynamic Measurements Obtained From Young (4 and 7 Weeks) and Adult (12 Weeks) Male Rats Exposed or Not to High Oxygen (O2) as Newborns (n=7 Rats per Group per Age)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (4 wk)</th>
<th>O2 (4 wk)</th>
<th>Control (7 wk)</th>
<th>O2 (7 wk)</th>
<th>Control (12 wk)</th>
<th>O2 (12 wk)</th>
<th>Age</th>
<th>O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail cuff</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>133±2</td>
<td>132±2</td>
<td>143±9</td>
<td>161±12*</td>
<td>138±24</td>
<td>164±17*</td>
<td>§</td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>483±5</td>
<td>483±8</td>
<td>463±24</td>
<td>486±12</td>
<td>445±26</td>
<td>481±45*</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>ECHO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>130±2</td>
<td>133±4</td>
<td>294±6</td>
<td>285±7</td>
<td>556±18</td>
<td>610±21</td>
<td>§</td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>357±9</td>
<td>361±11</td>
<td>360±10</td>
<td>351±7</td>
<td>331±7</td>
<td>323±10</td>
<td>‡</td>
<td></td>
</tr>
<tr>
<td>M-mode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>1.50±0.06</td>
<td>1.51±0.08</td>
<td>1.77±0.09</td>
<td>1.86±0.08</td>
<td>1.91±0.11</td>
<td>2.18±0.06*</td>
<td>† †</td>
<td></td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>5.64±0.14</td>
<td>5.91±0.21</td>
<td>6.52±0.20</td>
<td>6.80±0.23</td>
<td>7.74±0.13</td>
<td>8.16±0.22*</td>
<td>§</td>
<td></td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>1.45±0.06</td>
<td>1.54±0.04</td>
<td>1.93±0.08</td>
<td>2.13±0.13*</td>
<td>2.30±0.08</td>
<td>2.47±0.10*</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>LVWd, mm</td>
<td>3.01±0.14</td>
<td>3.60±0.22*</td>
<td>3.04±0.22</td>
<td>3.63±0.15*</td>
<td>4.19±0.14</td>
<td>4.57±0.23*</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>FS, %</td>
<td>47±2</td>
<td>37±2*</td>
<td>54±2</td>
<td>47±2*</td>
<td>49±2</td>
<td>44±2*</td>
<td>† †</td>
<td></td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>387±27</td>
<td>437±31</td>
<td>665±14</td>
<td>794±44*</td>
<td>1050±44</td>
<td>1317±32*</td>
<td>§ §</td>
<td></td>
</tr>
<tr>
<td>LV mass index, mg/g</td>
<td>3.0±0.2</td>
<td>3.3±0.2</td>
<td>2.3±0.1</td>
<td>2.8±0.2*</td>
<td>1.8±1</td>
<td>2.2±0.1*</td>
<td>§ §</td>
<td></td>
</tr>
</tbody>
</table>

Data presented are mean±SEM. A indicates mitral A wave; E, mitral E wave; ECHO, echocardiography; FS, fractional shortening; HR, heart rate; IVSd, interventricular septal thickness in diastole; LV, left ventricle; LVIDd, LV internal diameter in diastole; LVWd, LV internal diameter in systole; LVPWd, LV posterior wall thickness in diastole; and SBP, systolic blood pressure.

*P<0.05 compared with age-matched controls.
†P<0.05 when analyzing the effect of age or O2 exposure.
‡P<0.01.
§P<0.001 when analyzing the effect of age or O2 exposure.

Impact of Ang II Infusion on Hemodynamic Parameters and Cardiac Function

SBP (measured in the carotid under isoflurane anesthesia; Figure 6A) and the heart/body weight ratio (Figure 6B) similarly increased after 2 weeks of Ang II infusion (initiated at 12 weeks) in control and O2-exposed groups; no further increase was observed after 4 weeks of infusion. In saline-infused animals, there was no difference in SBP between groups, but heart/body weight ratios were higher in O2-exposed versus control animals after 2 or 4 weeks of infusion. LV end-diastolic pressure in control animals increased after 2 weeks of Ang II infusion without further increase after 4 weeks, whereas in O2-exposed animals LV end-diastolic pressures that are elevated at 4 weeks, only p53 was significantly increased and no difference between groups was noted for Rb (Figure 4A and 4B).
compared with control rats were not modified after 2 weeks but increased after 4 weeks of Ang II (Figure 6C). In control rats, +dP/dT and −dP/dT remained unchanged after 2 weeks of Ang II infusion and decreased after 4 weeks of Ang II infusion (Figure 6D and 6E). In O2-exposed rats, +dP/dT and −dP/dT increased after 2 weeks but were markedly reduced after 4 weeks of Ang II infusion to values lower than Ang II–infused control rats, indicating severe systolic and diastolic dysfunction and progression to heart failure.

**Discussion**

This study reveals that transient neonatal high O2 exposure resulted in structural and functional changes in the myocardium of young (4 weeks, before rise in BP) and adult rats with fibrosis, enhanced AT1 to AT2 receptor expression ratio, and upregulation of senescence-associated proteins. Adult O2-exposed rats also displayed an increased susceptibility to develop heart failure under Ang II infusion.

At 4 weeks, O2-exposed rats showed decreased FS (assessed by echocardiography), suggesting premature LV systolic dysfunction. These results are similar to changes observed in other experimental models of hypertension such as the genetic spontaneously hypertensive rat, in which LV dysfunction is also verified prior the BP increase. Dupont et al recently showed that 3-week-old spontaneously hypertensive rat developed LV systolic dysfunction with decreased FS and impaired LV diastolic function, suggesting early diastolic dysfunction. In addition, in spontaneously hypertensive rat, interstitial fibrosis and LV hypertrophy accompany BP elevation at an adult age. Similar to spontaneously hypertensive rat, where cardiomyopathy progresses from concentric to eccentric LV hypertrophy later in life (≈18 months),...
with systolic dysfunction and LV chamber dilation,\textsuperscript{18} in our model such transition occurs earlier at 12 weeks when dilated (eccentric) hypertrophy is accompanied by systolic and diastolic dysfunction.

To assess whether O\textsubscript{2} exposure predisposed rats to cardiovascular disease development, we used the well-known model of Ang II infusion.\textsuperscript{15} Under these conditions, both groups (control and O\textsubscript{2}-exposed) developed cardiac hypertrophy and elevated BP, whereas only O\textsubscript{2}-exposed animals showed severe systolic and diastolic dysfunction after 4 weeks of Ang II infusion. Interestingly, after 2 weeks of Ang II infusion, O\textsubscript{2}-exposed rats presented a significant increase in LV myocardial contractility and relaxation compared with control Ang II–infused animals, characteristic of a compensatory response. The mechanisms eliciting early compensatory response were not investigated in the current study but may include exacerbated fibrosis and myocardial hypertrophy. As we previously reported,\textsuperscript{6} BP of O\textsubscript{2}-exposed versus control animals was significantly increased when measured in conscious animals using tail-cuff and intra-arterial (femoral), but was not different under anesthesia. Current studies were not designed to undermine the reason why anesthesia would abrogate the BP difference between groups but one can postulate that specific mechanisms underlying developmentally programmed higher BP such as central nervous system/sympathetic activity and vascular function can be particularly attenuated by general anesthesia.\textsuperscript{14}

Few studies have described the developmental programming of cardiac dysfunction after different perinatal deleterious conditions. In a model of intrauterine growth retardation induced by intrauterine hypoxia and nutrient restriction during pregnancy, Xu et al\textsuperscript{19} reported LV hypertrophy and diastolic dysfunction.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Senescence-associated p53 and Rb proteins expression in 4- and 16-week-old rats exposed to high oxygen (O\textsubscript{2}) as newborns vs room air controls. Representative images and quantitative analysis of (A) p53 and (B) Rb proteins expression assessed by Western blotting in control and O\textsubscript{2}-exposed (O\textsubscript{2}) rats. *P<0.05 vs controls, n=4 to 6 rats per group, unpaired t test. Data presented are mean±SEM.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Angiotensin II type 1 (AT1) and AT2 protein and gene and angiotensin-converting enzyme (ACE) protein cardiac (left ventricle) expressions in 4- and 16-week-old rats exposed to high oxygen (O\textsubscript{2}) as newborns vs room air controls. AT1, AT2, and ACE protein expressions assessed by Western blotting, and AT1, AT2, and AT2 mRNA expressions by reverse transcriptase polymerase chain reaction. A, Representative images and quantification of AT1, AT2, and ACE receptor expression in controls and O\textsubscript{2}-exposed rats (O\textsubscript{2}). B, Compiled results for AT1, AT2, and AT2 mRNA expressions in controls and O\textsubscript{2} groups. *P<0.05 vs controls, n=6 rats per group, unpaired t test. Data presented are mean±SEM.}
\end{figure}
dysfunction at 4 and 7 months accompanied by increased ventricular fibrosis and increased susceptibility to ischemia/reperfusion injury. In this model, male offspring presented at birth larger hearts compared with controls although this difference tended to decrease at 4 months and then worsened at 12 months. In a mouse model the association of antenatal lipopolysaccharide–induced inflammation with neonatal hyperoxia also caused LV dysfunction at 8 weeks. Recently, Bensley et al assessed long-term (9 weeks) impact of preterm birth on the heart growth in sheep and observed increase in cardiomyocyte volume, collagen deposition, and alteration in cardiomyocyte maturation verified by increased nuclear ploidy; the impact on heart function was not reported in this model. These data support current results suggesting that cardiac changes occur prematurely in animals exposed to deleterious perinatal conditions that may translate into maladaptive responses to cardiovascular risk factors in the adult.

The RAS is one of the main mechanisms leading to cardiovascular and renal dysfunction in hypertension, and the most effective antihypertensive drugs antagonize this system. The present results reveal activation of the cardiac RAS by AT1/AT2 receptor imbalance after neonatal O2 exposure. Interestingly, these changes were present at 4 weeks, before significant BP elevation, with a significant decrease in AT2 protein and gene expressions and increased AT1b receptor mRNA expression although AT1 protein was not significantly different between groups. At 16 weeks, the imbalance is more evident in O2-exposed rats with important increases of AT1a and AT1b receptor protein and mRNA levels, and a sustained reduction in AT2 receptor protein and mRNA. AT2 receptor is involved in fetal development because of its high systemic expression in the fetus and its downregulation after birth. The role of the AT2 receptor in cardiovascular development was described in AT2 receptor–deficient mice that develop at very early ages (7 and 14 days) increased both body and heart weight although the heart/body weight ratio is decreased. At 8 weeks, however, these mice show myocardial hypertrophy and increased heart/body weight ratio.

AT1 receptors are involved in the maturation and growth of the heart during fetal and postnatal development, and the use of AT1 blockers during pregnancy is associated with cardiac malformations. However, persistent upregulation of AT1 receptors later in life can lead to myocardial hypertrophy and changes in contractility. In our study, upregulation of AT1 receptors could contribute to the development of cardiac remodeling and to cardiac compensating/decompensating responses during Ang II infusion in O2-exposed rats. Supporting a role for early upregulation of AT1 expression in cardiac remodeling, transgenic mice overexpressing AT1 receptor specifically in cardiomyocytes also develop myocardial hypertrophy, fibrosis, and progression to congestive heart failure as adults (4–5-month); cardiac changes in these transgenic mice also occur independently of changes in BP. Using the same transgenic model, Rivard et al also showed that increased AT1 receptor is associated with an early decrease in systolic function. More recently, Yasuda et al have generated the same transgenic mice in angiotensinogen-knockout background and showed that upregulation of AT1 receptor in the heart leads to cardiac remodeling even in the absence of Ang II. AT1 receptors are also known to stimulate cardiac fibrosis, in
part, via the TGF-β1 pathway. This study shows myocardial fibrosis early, at 4 weeks, as well as in adults; however, TGF-β1 expression was increased in adults only. It could be postulated that in adults, increased expression of TGF-β1 could be secondary to higher BP in O2-exposed rats and thereby contributes to the development of LV diastolic dysfunction. Moreover, the profibrotic mechanism and also oxygen-sensitive HIF-1α is upregulated before the elevation of BP and seems to be a potential mechanism stimulating early fibrosis in O2-exposed rats. In addition, cross-talk between Ang II/AT1 receptor and HIF-1α was previously described in human pulmonary artery fibroblasts, suggesting HIF-1α as a downstream factor stimulated by Ang II–activating profibrosis pathways. The current studies also show increased expression of senescence-associated proteins p53 and Rb in O2-exposed rats. In the heart, the aging process is characterized by increased interstitial and perivascular fibrosis, as well as cardiomyocyte hypertrophy, a process accelerated in the presence of increased cardiac afterload such as hypertension, ultimately leading to heart failure. In humans, few studies have examined cardiac consequences of perinatal deleterious conditions.

Epidemiological studies have highlighted a geographic relationship between rates of infant mortality (a surrogate marker of low birth weight) and the incidence of ischemic heart disease measured. Barker et al also noted a link between impaired placental growth (which leads to fetal malnutrition and low birth weight) and adult heart failure. Low birth weight has been associated with reductions in total cardiac diameter and LV outflow tract in 9-year-olds and with concentric ventricular hypertrophy in adults. To our knowledge, however, very few studies have evaluated the cardiac repercussions of preterm birth itself. Mikkola et al reported increased interventricular septum thickness and decreased LV end-diastolic diameter in 5-year-olds born at 28 weeks of gestation as compared with values of a reference population. Thus, current data corroborate clinical and epidemiological data suggesting that neonatal high O2 exposure in an immature individual could be a risk factor for cardiac disease at adult age.

In conclusion, the current study highlights the existence of early cardiac dysfunction in young and adult rats exposed to high O2 as newborns and the increased susceptibility to develop heart failure under Ang II infusion in the adult. Importantly, cardiac structural and functional alterations occur before the onset of high BP and are associated with an early and sustained increase in AT1/AT2 receptor expression ratio, a candidate mechanism contributing to the prohypertrophic, profibrotic, and senescence-associated changes observed in the hearts of O2-exposed rats.

Perspectives

Although the association among oxidative stress, cardiovascular disease, and aging is well known, the fact that a transient exposure to O2-related oxidative conditions in the newborn period can lead to changes in myocardial tissue, perpetuated to adulthood, and leading to conditions such as heart failure is to our knowledge a novel concept which had not been reported. Considering that fetal/neonatal deleterious conditions can have differing developmental programming effects on cardiovascular diseases and dysfunction between males and females, in experimental models and in humans (including preterm born individuals), and that pathways and mechanisms to adult diseases can also differ between sexes, one cannot indistinctly transpose current findings to females. Nevertheless, these results are of major importance for the growing population of former prematurely born individuals, the first generation of survivors now reaching their 30s to 40s. Recognition that former premature infants carry an increased cardiovascular risk is particularly important because they might often not present classical risk factors, such as obesity and dyslipidemia, and familial risk factors might not be present.

Acknowledgments

We thank Keven Younide (Université de Sherbrooke) for the technical support with the experimental procedures, Alexandre Barbier, and Catherine Zydorczyk for helpful suggestions.

Sources of Funding

E.L. Schiffrin was supported by Heart and Stroke Foundation of Canada (Quebec) Grants-in-aid, Canadian Institute of Health Research (CIHR) grants MOP106554 and MOP123465, a Canada Research Chair from the CIHR/Government of Canada Program, and a Canada Fund for Innovation. M. Bertagnolli was supported by “Bourse d’excellence pour étudiants étrangers du ministère de l’Éducation, du Loisir et du Sport du Québec.” Fonds québécois de la recherche sur la nature et les technologies” and by a Jacques-de Champlain-Fonds de la recherche en santé du Québec fellowship award. A.M. Nuyt was supported by a salary award from the Fonds de la Recherche en Santé du Québec.

Disclosures

None.

References

Neonatal high O2 exposure in rats leads to :

- Cardiac fibrosis, hypertension, changes in renin–angiotensin system components (increase angiotensin II type 1/angiotensin II type 2), and increased expression of senescence-associated proteins perpetuated to adulthood.
- Impaired cardiac responses to hemodynamic stress in adults.

What Is Relevant?

- Neonatal high O2 exposure in immature/susceptible subjects (as encountered in preterm infants) could be a newly identified risk factor for early cardiac dysfunction.

Neonatal high O2 exposure leads to cardiac remodeling, fibrosis, and dysfunction. This study is relevant for the growing proportion of adults born preterm who might be at higher risk of cardiac dysfunction when faced with increased vascular resistance associated with hypertension, vascular diseases, or (premature) aging.

Novelty and Significance

What Is New?

- Cardiac fibrosis, hypertension, changes in renin–angiotensin system components, and impaired cardiac responses to hemodynamic stress in adults.

Summary

Neonatal high O2 exposure leads to cardiac remodeling, fibrosis, and dysfunction. This study is relevant for the growing proportion of adults born preterm who might be at higher risk of cardiac dysfunction when faced with increased vascular resistance associated with hypertension, vascular diseases, or (premature) aging.
Transient Neonatal High Oxygen Exposure Leads to Early Adult Cardiac Dysfunction, Remodeling, and Activation of the Renin–Angiotensin System

Mariane Bertagnolli, Fanny Huyard, Anik Cloutier, Zackary Anstey, Julie-Émilie Huot-Marchand, Catherine Fallaha, Pierre Paradis, Ernesto L. Schiffrin, Denis deBlois and Anne Monique Nuyt

_Hypertension_. 2014;63:143-150; originally published online October 28, 2013; doi: 10.1161/HYPERTENSIONAHA.113.01760

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/63/1/143

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2013/10/28/HYPERTENSIONAHA.113.01760.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/
ONLINE SUPPLEMENT

TRANSIENT NEONATAL HIGH OXYGEN EXPOSURE LEADS TO EARLY ADULT CARDIAC DYSFUNCTION, REMODELING AND ACTIVATION OF THE RENIN-ANGIOTENSIN SYSTEM

Mariane Bertagnolli, Ph.D. 1, Fanny Huyard, M.Sc. 1; Anik Cloutier, M.Sc. 1; Zackary Anstey, M.Sc. 1; Julie-Émilie Huot-Marchand, B.Sc. 2; Catherine Fallaha 1; Pierre Paradis, Ph.D. 4; Ernesto L. Schiffrrin, M.D., Ph.D. 4; Denis deBlois, Ph.D. 2,3; Anne Monique Nuyt, M.D. 1

1Department of Pediatrics, Sainte-Justine University Hospital Research Center, Université de Montréal, Montreal, Quebec, Canada
2Department of Pharmacology, Université de Montréal, Montreal, Quebec, Canada
3Faculty of Pharmacy, Université de Montréal, Montreal, Quebec, Canada
4Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University, Montreal, Quebec, Canada

Corresponding Author:

Anne Monique Nuyt, M.D.

Division of Neonatology, Department of Pediatrics
Sainte-Justine University Hospital Research Center
3175, Chemin de la Côte-Sainte-Catherine
H3T 1C5, Montreal, Quebec, Canada
Telephone number: 1(514)345-4931 x3971
Fax number: 1(514)345-4999
Email: anne-monique.nuyt@recherche-ste-justine.qc.ca
SUPPLEMENTAL MATERIALS AND METHODS

Echocardiography

Echocardiography analyses were performed using ACUSON CV70 Ultrasound Imaging System (Siemens Medical Solutions, Burlington, ON) with a scan head of 12 MHz in rats at 4, 7 and 12-week old under inhaled isoflurane anesthesia. All procedures follow the guidelines of the American Society of Echocardiography. Cardiac hypertrophy and left ventricular (LV) systolic function were assessed as previously described by M-mode two-dimensional images in the paraesternal long and short-axis\(^1\). The dimensions of the LV internal diameter and the thickness of the walls were obtained using a left parasternal short-axis view of the heart. LV systolic function was evaluated by the ejection and shortening fractions obtained on M-mode of the LV short-axis. Ventricular diastolic function was assessed on transmitral Doppler signal by measuring E and A wave velocities and gradients and calculating E/A index and mitral deceleration time.

Intraarterial Blood Pressure and in vivo Intraventricular Pressures and Derivatives

To assess intraarterial blood pressure (BP), rats at 16-week old (previously assessed by echocardiography at 4, 7 and 12-week old) were anaesthetized with inhaled isoflurane and placed in controlled heating pads. Under normoxic conditions, a polyethylene PE10 catheter (I.D. 0.28 mm, Becton Dickinson and Company, Franklin Lakes, NJ) was inserted into the femoral artery and placed in the dorsal area to allow free mobility in the cage. The rats were then allowed 24 hours to recover from the surgical procedure and BP was recorded during continuous 30 minutes (i.e. in conscious animals). To assess intraventricular pressures in rats previously recorded and in rats from Ang II or saline infusion experiment (at 14 and 16-week old after respectively 2 and 4 weeks of infusion), rats were anaesthetized with inhaled isoflurane and a PE50 catheter (I.D. 0.58 mm, Becton Dickinson and Company, Franklin Lakes, NJ, United States) was inserted into the right carotid and advanced into the LV to obtain LV systolic and end-diastolic pressures, as well as maximum and minimum derivatives (+ and -dP/dT). Intraarterial (carotid) and intraventricular signals were continuously recorded during 10 minutes by a signal amplifier (P122 AC/DC Strain Gage Amplifier, Grass Technologies, West Warwick, RI) and analysed using PolyVIEW16 software (Grass Technologies, West Warwick, RI). Rats were then sacrificed under anaesthesia effect by decapitation and hearts dissected.

Histological Analysis

Hearts were rapidly removed and washed 3 times in KCl (100 mM) to induce diastolic arrest. Ventricles were isolated together, weighted and fixed during 24-48 hours at 4\% paraformaldehyde. Equatorial cross-sections were paraffin-embedded and 5 µm sections stained with hematoxylin and eosin for the measurement of cardiomyocyte surface area, cell counting and cell volume. In addition, ventricular sections (5 µm) were stained with Masson’s Trichrome to evaluate cardiac fibrosis. For all histological analyses, three pictures were obtained randomly each from the sub-endocardium, the sub-epicardium and the mid-myocardium of the LV. Cardiomyocyte size was evaluated in the sub-endocardium and sub-epicardium by measuring the cross-sectional of cells with a visible nucleus and a perpendicular orientation relative to the plane. The total number of cardiomyocyte nuclei in the left ventricle was evaluated stereologically as we described previously\(^2\). Cardiac fibrosis was assessed by quantifying the blue in pixels (corrected as % of the total) obtained from the Masson’s trichrome staining. The software
ImageJ 1.36b (http://rsbweb.nih.gov/ij/) was used for stereological analysis, as well as pixels quantification as previously described\textsuperscript{2,3}.

**Western Blotting**

Hearts were homogenized in RIPA buffer containing protease inhibitors. Antibodies against AT1 (1/1000 dilution, Abcam, Cambridge, MA) and AT2 receptors, angiotensin-converting enzyme (ACE), p53, HIF-1α, Rb (1/1000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA), and TGF-β1 (1/1000 dilution, Abcam, Cambridge, MA) were used in this study. Antibody against β-tubulin (1/2500 dilution, Sigma-Aldrich Canada Co., Oakville, ON) was used as control. Protein bands were developed with an enhanced chemiluminescence substrate (PerkinElmer Inc, Waltham, MA) and quantified using ImageJ 1.36b (http://rsbweb.nih.gov/ij/) with values normalized against β-tubulin expression.

**Semi-Quantitative Reverse Transcription and Real-Time PCR**

Total RNA was extracted from hearts using RNeasy Mini Kit (Qiagen Inc, Toronto, ON). RNA samples (1 µg) were treated with DNase to eliminate genomic DNA in the samples. mRNA expression was assessed by polymerase chain reaction after reverse transcription of RNA. Total RNA treated with DNase was reversing transcribed using Omniscript RT Kit (Qiagen Inc, Toronto, ON). Fragments of single-stranded cDNA were amplified by PCR using SYBER Green PCR Master Mix (Applied Biosystems, Carlsbad, CA) and performing 45 cycles (10 min 95°C; 15 s 95°C; 1 min 60°C). The following genes were amplified using specific primers: AT1a (forward 5’-CCAAAGTCCCACATCAAGCCT-3’ and reverse 5’-TTGCCAGTGGCTTTGAACC-3’), AT1b (forward 5’-GCACCTTTTCTACCGCCCTC-3’ and reverse 5’-CACTTTCTCTGTCAACCCTG-3’) and AT2 (forward 5’-TGTGTTGCGATTCAATCATTG-3’ and reverse 5’-AGAACTGCTTTTTCGGCAAG-3’). The endogenous β-actin was used as internal control (forward 5’-ATTGTCACCACTGGGACGATA-3 and reverse 5’-GGCTGCGGGTGGTTGAAGGTCT-3’).

**Statistical Analysis**

Data are presented as mean ± SEM in the Table and Figures. Analyses of echocardiography and tail-cuff BP data are performed using two-way ANOVA for repeated measures followed by Bonferroni post-test, considering the age (4, 7 and 12-week old) and oxygen exposure (O\textsubscript{2}-exposed vs. control) as factors. The same test was used to analyse data from AngII or saline infusion, where oxygen-exposure and AngII infusion were the factors. Student t test was used for the additional data analysis comparing control and oxygen-exposed groups (data 4- and 16-weeks old). The software GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, United States) was used for all tests performed. The significance level is established at P<0.05.

**ONLINE SUPPLEMENT REFERENCES**
