Effects of neonatal hypoxia in the rat on inotropic stimulation of the adult heart

Charles V. Rohlicek a,*, Stephanie Viau a, Phan Trieu b, Terence E. Hébert b

a Division of Cardiology – Department of Pediatrics, McGill University, Canada
b Montreal Heart Institute Research Center, Canada

Received 22 June 2004; received in revised form 18 November 2004; accepted 6 December 2004
Available online 7 January 2005
Time for primary review 36 days

Abstract

Objective: To determine whether transient hypoxia in neonatal rats has long-lasting effects on inotropic stimulation of the adult heart.

Methods: The hearts of adult male Sprague–Dawley rats (89 ± 1 (S.E.M.) days, 432 ± 5 g) were studied. Half the animals had been subjected to neonatal hypoxia (FiO2 = 0.12, days 1–10) while the others had not. The peak response of left ventricular pressure (LVP) and the maximum rate of pressure increase (+dP/dt max) were measured in isolated and perfused hearts during application of dobutamine, isoproterenol, milrinone and betaxolol. Left ventricular myocyte membranes were analyzed for β receptor density, adenylyl cyclase activity and content.

Results: LVP and +dP/dt max responses to stimulation with dobutamine and isoproterenol were significantly impaired in adult hearts of neonatally hypoxic rats. The inotropic effect of dobutamine was abolished by blockade with the β1-selective antagonist betaxolol. The inotropic effects of the phosphodiesterase inhibitor milrinone were also significantly decreased in neonatally hypoxic adult hearts. There was no difference in left ventricular myocyte membrane β receptor density of adult hearts whether they were hypoxic neonatally or not. However, left ventricular adenylyl cyclase activity on isoproterenol or forskolin stimulation and adenylyl cyclase levels (type V/VI) were significantly reduced in neonatally hypoxic adult hearts.

Conclusions: Neonatal hypoxia in the rat has long-lasting effects on the left ventricular response to inotropic stimulation at maturity likely at least in part due to diminished left ventricular adenylyl cyclase levels.

© 2004 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Hypoxia/anoxia; Receptors; Contractile function; Inotropic agents; Signal transduction

1. Introduction

Prolonged hypoxemia during infancy is frequently the result of a congenital cyanotic heart defect or lung disease resulting from pre-maturity. Hypoxemia may last several weeks or months in such situations until surgical repair of the heart malformation or improvement in pulmonary function take place. Prior studies have revealed that respiratory as well as cardiovascular function and control at maturity may be abnormal after hypoxemia experienced in early life [1–3]. The mechanisms responsible for such long-lasting effects of hypoxic exposure in early life remain unclear. Although central nervous control systems may be altered following chronic neonatal hypoxia, it is also possible that there are long-term effects on peripheral effector responsiveness. With respect to the heart, this might result from changes in myocardial adrenergic signal transduction.

Chronic hypoxia results in a lower resting heart rate and a blunted cardiac responsiveness to β-adrenergic stimulation [4]. Previous investigations have shown that β-adrenergic receptor density and isoproterenol stimulated adenylyl cyclase activity in left ventricular myocardium is decreased following chronic hypoxia in adult rats [5–8] as well as in
chronically hypoxic newborn sheep [9]. Whether the decreased β agonist response can be entirely accounted for by a decrease in β-adrenergic receptor density is unclear. Experiments in adult rats [6] and in newborn sheep [10] made chronically hypoxic have indicated that the left ventricular cAMP generation in response to direct G-protein stimulation with NaF or adenylyl cyclase stimulation with forskolin is unchanged. However, more recent experiments on left ventricular myocardium of chronically hypoxic adult rats have demonstrated a decrease in adenylyl cyclase activation by NaF [8,11] and forskolin [7,8,11]. Furthermore, it has been shown that chronic hypoxia in the adult rat results in decreased functional Gsa activity in the left ventricle [8,11] although mRNA and protein levels are unaffected [6,11].

Of interest is the work of Quaeghebeur and colleagues [12,13] concerning right ventricular infundibular tissue from patients with Tetralogy of Fallot. Individuals with this cardiac malformation are often hypoxemic at rest and are prone to paroxysmal episodes of severe hypoxemia, which may be at least partly due to right ventricular infundibular hypercontractility. These investigators have demonstrated an increase in right ventricular β receptor density and iso-proterenol-stimulated adenylyl cyclase production in patients with Tetralogy of Fallot symptomatic with hypercyanotic episodes compared to those without such episodes [12]. More recently they have shown that right ventricular adenylyl cyclase types V and VI mRNA and protein expression are greater in patients with Tetralogy of Fallot exhibiting cyanosis compared to those without cyanosis [13]. It is not clear whether these changes are due to the patient’s hypoxemia or related to their cardiac malformation.

There has been limited study concerning the long-term effects of hypoxia on myocardial β-adrenergic responsiveness and the β-adrenergic/adenylyl cyclase signalling system. Hrabosova et al. [14] have recently described the effects of simulated high altitude acclimatisation and return to normoxia on right ventricular contractile function, left and right ventricular β-adrenergic content, G protein content and function, as well as on adenylyl cyclase activation in adult rats. They have found that 5 weeks of progressive intermittent hypobaric hypoxia resulted in a decreased inotropic response to isoproterenol of the isolated and perfused right ventricle, which returned to normal at 5 weeks following the return to normoxia. Right and left ventricular β-adrenergic receptor density, membrane bound Gsa as well as Gia content were unchanged by hypoxia or following the return to normoxia. However, Gsa activity, as well as adenylyl cyclase activation by GTP, sodium fluoride, forskolin, and isoproterenol decreased with hypobaric hypoxic exposure in both ventricles and remained decreased in the right but not the left ventricle at 5 weeks following the return to normoxia.

There has been no previous investigation of the long-term effects of chronic neonatal hypoxia on left ventricular responsiveness to β-adrenergic stimulation or adenylyl cyclase signal transduction at maturity. This could be of clinical significance. For instance, a long-lasting decrease in β-adrenergic responsiveness of the ventricular myocardium following neonatal hypoxia might limit the ability of individuals hypoxic in early life to initiate rapid autonomically mediated adjustments in ventricular function. A decrease in β-adrenergic responsiveness might also become significant following cardiac surgery if pharmacologic inotropic support is required. In this regard we have studied the left ventricular contractile response to β-adrenergic stimulation in the isolated and perfused hearts of adult rats made hypoxic for 10 days neonatally and compared these responses to those obtained in hearts from adult rats never made hypoxic. We have further characterized the effects of neonatal hypoxia on adult left ventricular β receptor density, as well as on isoproterenol-, NaF-, and forskolin-stimulated adenylyl cyclase activity, and on protein levels for adenylyl cyclase type V/VI. The hypothesis tested is that hypoxic exposure in early life alters ventricular β-adrenergic signal transduction at various levels in the adult heart.

2. Methods

2.1. Animals

Experiments were conducted on the hearts of 112 adult male Sprague–Dawley rats (89 ± 1 (S.E.M.) days, 432 ± 5 g). Fifty-six animals had experienced hypoxia (FiO2=0.12) continuously along with their mothers from day 1 to day 10 of life (neonatally hypoxic). The size of the litters made hypoxic was decreased by removing all female offspring on day 2 of life. Following the period of chronic hypoxia, the rats were reared in normoxia. The other 56 male rats were not made hypoxic prior to the experiments outlined below (control).

2.2. Isolated and perfused hearts

The hearts of 35 adult animals of each group were isolated and perfused in the Langendorff mode. Following anesthesia with pentobarbital (65 mg/kg ip), the hearts were exposed, chilled with cold saline, and cannulated in situ by way of the aorta. The hearts were perfused at a constant pressure of 100 cm H2O with modified Krebs-Ringer buffer at 37 °C consisting (in mM) of 119 NaCl, 4.8 KCl, 1.3 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3 as well as 15 glucose maintained at 37 °C and aerated with 95% O2+5% CO2. A small balloon was inserted in the left ventricle by way of the mitral valve. This balloon was connected to a pressure transducer and its volume adjusted to achieve a diastolic pressure of 10 mm Hg at the beginning of the experiment and the volume kept constant for the duration of the study. The pressure signal was recorded digitally using Powerlab instrumentation and software in conjunction with a Macintosh Powerbook 180 computer. Heart rate (HR),
peak left ventricular developed pressure (LVP), and the maximum rate of pressure increase (+dP/dt max) were continuously monitored. Following 30 min of perfusion heart rate, LVP, and +dP/dt max were noted and dobutamine (Sabex), isoproterenol (Sabex), betaxolol (Tocris), or milrinone (Sanoﬁ-Synthelabo) were added to the perfusate as outlined below. Heart rate, LVP, and +dP/dt max were again noted 10 min following the addition of each drug or change in drug concentration.

In ten animals of each group, dobutamine was sequentially added every 10 min to the perfusate to achieve concentrations of 10⁻⁷ M, 2×10⁻⁷ M, 10⁻⁶ M and 10⁻⁵ M. In ten further animals from each group, isoproterenol was added to the perfusate to achieve a concentration of 4×10⁻⁶ M. In five additional animals of each group, betaxolol was added to the perfusate to produce a concentration of 10⁻⁵ M prior to the addition of dobutamine 10⁻⁵ M. In ten other animals of each group, milrinone was added to the perfusate to yield a concentration of 4.7×10⁻⁵ M.

2.3. Myocardial membrane preparation

Myocardial membranes were prepared from left ventricular tissue of 21 additional control and 21 additional neonatally hypoxic adult animals as described previously [15]. Frozen myocardial tissue was washed twice with ice-cold PBS. Myocardial tissue was then disrupted by homogenization with a polytron (2×15 s bursts) in 10 ml of ice-cold buffer containing 5 mM Tris–HCl, pH 7.4, 2 mM EDTA, 5 µg/ml leupeptin, 10 µg/ml benzamidine and 5 µg/ml soybean trypsin inhibitor. Lysates were centrifuged at 45,000 g for 10 min at 4 °C, and the supernatant was then centrifuged at 45,000×g for 20 min and the pellet washed twice in the same buffer. This membrane preparation was then immediately used for adenylyl cyclase and binding assays or for Western blotting as described below.

2.4. β-receptor quantification and adenylyl cyclase assay

Total left ventricular β-adrenergic receptor number was calculated from binding experiments in 5 animals from each group using [¹²⁵I] cyanopindolol (CYP) as the radioligand. Membrane preparations (250 µg of protein) in a total volume of 0.5 ml were labeled with 230 pM [¹²⁵I]-CYP. This represents a near saturating concentration of CYP. Non-specific binding was defined using 10 µM alprenolol. Adenylyl cyclase activity was assayed by the method of Salomon et al. [16]. Membranes were prepared from 12 additional animals from each group as described above. Again, 20 µL of membranes (20 µg of membrane protein) was used in a total volume of 50 µL. Enzyme activities were determined in the presence of 1 µM isoproterenol, 100 µM forskolin or 10 mM NaF. Data were calculated as pmoles cAMP produced/min/mg protein. All receptor binding and adenylyl cyclase assays were performed in triplicate.

2.5. SDS PAGE and Western blotting

Membrane protein from four animals of each group (25, 50 or 75 mg) was heated for 15 min at 65 °C and loaded into lanes of a 10% SDS polyacrylamide gel. After separation, gels were transferred to a nitrocellulose membrane. Membranes were incubated in 5% nonfat dry milk in PBS (pH 7.5) supplemented with 0.05% Tween-20 (PBST) for 2 h at room temperature, followed by incubation with primary anti-adenylyl cyclase V/VI antibody (Santa Cruz, 1:1000 dilution), anti-adenylyl cyclase II antibody (Santa Cruz, 1:1000 dilution) or anti-GRK2 antibody (Santa Cruz, 1:1000 dilution) in PBST at 4 °C overnight. Following removal of the primary antibody, membranes were washed (3×10 min) with PBST and incubated with horseradish peroxidase-conjugated affinity purified goat anti-rabbit secondary antibody (Sigma, 1:20,000 dilution) in PBST containing 5% nonfat dry milk for 2 h at room temperature. Membranes were again washed (3×10 min) with PBST and immune complexes visualized by enhanced chemiluminescence (Perkin-Elmer Life Sciences).

2.6. Statistics

All data are expressed as mean±S.E.M. Data concerning age, weight, and heart mass as well as LVP and +dP/dt max during drug stimulation as a percentage of baseline levels, left ventricular β-receptor content, and left ventricular adenylyl cyclase activity in the two groups of rats (control and neonatally hypoxic) were compared using an independent t-test. Where multiple drug concentrations were used the percentage change from baseline in LVP and +dP/dt max were compared using analysis of variance with repeated measures for 2 factors [(i) drug concentration, (ii) pretreatment (i.e. control and neonatally hypoxic)]. Contrasts were subsequently made at drug concentrations where a significant interaction between drug concentration and pretreatment was found. The null hypothesis of no effect was rejected at P<0.05.

The experimental protocol was reviewed and approved by the Montréal Children’s Hospital–McGill University Research Institute animal care committee. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

3. Results

3.1. Age, body weight, heart weight

There was no difference in the mean ages of the 56 control and 56 neonatally hypoxic adult rats (88±2 vs. 92±2 days) used in this study. The mean body weight of the adult control animals (444±6 g) was significantly greater than that of the adult neonatally hypoxic rats (419±8 g). In
contrast, the hearts of the latter animals were on average significantly heavier than those of the control rats (2.01±0.06 g vs. 1.82±0.03 g). The greater mass of the neonatally hypoxic adult hearts compared to the control adult hearts was due both to a greater right ventricular free wall mass (0.42±0.03 g vs. 0.29±0.02 g, P<0.05) as well as to a greater left ventricular mass (1.19±0.05 g vs. 0.98±0.03 g, P<0.05).

3.2. Inotropic effects of dobutamine

Under baseline conditions, there was no difference between control (n=10) and neonatally hypoxic (n=10) adult hearts in peak left ventricular developed pressure (LVP) (84±4 vs. 84±3 mm Hg), +dP/dtmax (1626±84 vs. 1638±65 mm Hg/s), or heart rate (290±10 vs. 312±11 beats/min). Sequential addition of dobutamine to the perfusate to achieve concentrations of 10⁻⁷ M, 2×10⁻⁷ M, 10⁻⁶ M and 10⁻⁵ M increased LVP and +dP/dtmax in the hearts of both control and neonatally hypoxic adult rats (see Fig. 1). The latter increases were significantly less in the neonatally hypoxic adult hearts compared to the control adult hearts at concentrations of infused dobutamine of 10⁻⁶ M and 10⁻⁵ M. Although the infusion of dobutamine was associated with increases in heart rate, there was no significant differences in heart rate between the two groups of hearts at any of the concentrations of dobutamine infused.

3.3. Inotropic effects of isoproterenol and effect of selective β₁ blockade on dobutamine stimulation

In order to demonstrate that the effects observed during dobutamine stimulation were due to β-adrenergic stimulation, the experiments were repeated using the nonselective adrenergic agonist isoproterenol as well as following selective β₁ adrenergic blockade. Addition of isoproterenol to achieve a concentration of 4×10⁻⁶ M was associated with a significantly greater increase in LVP and +dP/dtmax in the control adult hearts (n=10) compared to the neonatally hypoxic adult hearts (n=10) (LVP; 65±3% vs. 54±4%, +dP/dtmax; 76±5% vs. 60±4%). Addition of the β₁-selective antagonist betaxolol to the perfusate to produce a final concentration of 10⁻⁶ M completely prevented the increases in LVP and +dP/dtmax in response to 10⁻⁵ M dobutamine in both control (n=5) and neonatally hypoxic (n=5) adult hearts demonstrating that this receptor subtype is likely responsible for the stimulation of cardiac contractility by dobutamine.

3.4. Inotropic effects of milrinone

To determine whether the mechanism of impaired adrenergic stimulation of the left ventricle was entirely at the level of β-adrenergic receptor/agonist interaction, the left ventricular contractile response of the isolated and perfused heart was studied during the addition of the phosphodiesterase inhibitor milrinone to the perfusate. When milrinone was added to the perfusate to yield a concentration of 4.7×10⁻⁵ M, a significantly greater increase in LVP and +dP/dtmax was observed in the control adult hearts (n=10) compared to the neonatally hypoxic adult hearts (n=10) (see Fig. 2).

3.5. Signalling deficits in neonatally hypoxic animals

In order to determine the mechanistic basis for the defect in β₁ adrenergic-mediated increases in left ventricular contractility, we assessed the total density of β₁-adrenergic receptors, adenylyl cyclase content and activity in left ventricular myocardial membrane preparations from control and neonatally hypoxic adult hearts. At saturating concentrations of labeled ligand, there was no difference in the β-receptor content of left ventricular tissue of control adult hearts (0.04±0.02 pmol/mg, n=5) and neonatally hypoxic adult hearts (0.03±0.01 pmol/mg, n=5).

To assess downstream signaling events, the adenylyl cyclase activity of left ventricular myocardial membranes

---

**Fig. 1. (a) Peak left ventricular developed pressure (LVP) and (b) maximum rate of pressure increase (+dP/dtmax) as a percentage of baseline levels (vertical axes) in isolated and perfused hearts of adult control rats (n=10) and of adult rats which were neonatally hypoxic (n=10) in response to stimulation with dobutamine (µM; horizontal axes). Values shown are mean±S.E.M with the best lines fitted by interpolation. Significant differences are indicated (*P<0.05).**
was determined in tissue from 12 control and 12 neonatally hypoxic adult rats. There was no difference in basal adenylyl cyclase activity of tissue from control or neonatally hypoxic adult rats. However, cAMP production in response to application of isoproterenol or forskolin was significantly less in tissue from neonatally hypoxic adult rats compared to control adult animals. There was no significant difference in NaF-stimulated adenylyl cyclase activity of left ventricular myocardial membranes from control and neonatally hypoxic adult rats suggesting that the balance between Gs and Gi was unaltered in these animals (see Fig. 3). Another possible explanation for decreased receptor-stimulated AC activity could be upregulation of G protein-coupled receptor kinase (GRK) activity responsible for desensitizing the receptor (see [17] for review). However, determination of GRK2 levels by Western blot analysis in control and neonatally hypoxic left ventricle revealed only modest differences in expression of this enzyme (data not shown).

To confirm that the deficit in β-adrenergic receptor-mediated signaling was at the level of the effector, adenylyl cyclase we used Western blot analysis. Our data indicated that the predominant cardiac isoforms of adenylyl cyclase (type V/VI) were markedly reduced in the left ventricular myocardium of neonatally hypoxic adult rats compared to control adult animals (Fig. 4). It could be argued that the reduction in cAMP formation was not as dramatic as the reduction in levels of AC (type V/VI). It is possible that other isoforms of AC may have been upregulated to compensate. In this regard Western blot analysis for AC (type II) indicated only a slight increase in left ventricular levels of this isoform (data not shown).

4. Discussion

We have shown that the effectiveness of β-adrenergic stimulation of the adult left ventricular myocardium is decreased following neonatal hypoxia. This is associated with reduced myocardial adenylyl cyclase (type V/VI). To our knowledge such a long-lasting alteration in β-adrenergic signal transduction following neonatal hypoxia has not been previously described.

Our results contrast with those of Hrbasova et al. [14] who did not find any long-lasting effect of 5 weeks of progressive intermittent hypobaric hypoxia in adult rats on left ventricular adenylyl cyclase activation by forskolin, isoproterenol or fluoride. These investigators did not study the contractile function of the left ventricle following hypoxic exposure. They did observe impaired adenylyl cyclase activation in the right ventricle 5 weeks following a return to normoxia. However, the right ventricular contractile response to isoproterenol stimulation was unaffected by prior hypoxia. It is quite likely that the discrepancies from our own results are related to the significant differences in experimental protocol, the different developmental states of the animals at the time of hypoxic exposure and the different types of hypoxic exposure utilized.

Fig. 2. (a) Peak left ventricular developed pressure (LVP) and (b) maximum rate of pressure increase (+dP/dtmax) as a percentage of baseline levels (vertical axes) in isolated and perfused hearts of adult control rats (n=10) and of adult rats hypoxic neonatally (n=10) (horizontal axis) in response to addition of milrinone (4.7x10^-5 M) to the perfusate. Values shown are mean±S.E.M. Significant differences are indicated (*P<0.05).

Fig. 3. Adenylyl cyclase activity of left ventricular myocardial membranes (pmol/min/mg protein) in 12 control (C) and 12 neonatally hypoxic (NH) rats under basal conditions as well as in response to isoproterenol, NaF, and forskolin. Values shown are mean±S.E.M. Significant differences are indicated (*P<0.05).

Fig. 4. Representative Western blot analysis of adenylyl cyclase type V/VI content in left ventricular myocardium of 3 adult control rats (left) and 3 adult animals which were neonatally hypoxic (right). Each lane represents membrane protein isolated from left ventricle of individual animals. Data are representative of five such blots and protein loading was controlled for by using multiple concentrations of membrane protein in both conditions. Molecular weight markers are shown to the left of each gel and adenylyl cyclase is denoted with an arrow.
In a previous series of experiments in awake adult rats, we showed that in contrast to control animals, cardiac output increased significantly during acute hypoxia in adult rats that had been exposed to chronic hypoxia neonatally [3]. The results presented here indicate that this increase in cardiac output was not due to left ventricular adrenergic hyper-responsiveness. Rather, the increased cardiac output response to acute hypoxia may have been necessitated by the greater fall in total peripheral vascular resistance in order to maintain systemic arterial pressure in the rats that had suffered chronic hypoxia neonatally [3]. This may have been due to impaired adrenergic responsiveness of the peripheral vasculature in a fashion similar to that which we have demonstrated here for the left ventricular myocardium.

The body mass of the neonatally hypoxic adult rats was significantly less than that of the control animals. This is in agreement with the data of Sant’Anna [18] who found that rats experiencing hypobaric hypoxia during the first week of life had a significantly lower body weight at 90 days of age compared to control animals. We have also found that cardiac mass was increased in adult rats made transiently hypoxic neonatally. A large portion of this increased mass was due to right ventricular hypertrophy with a smaller portion due to left ventricular hypertrophy. The presence of right ventricular hypertrophy following neonatal hypoxia is consistent with our previous investigations [3] as well as with that of others [19–21] who have also noted a persistent right ventricular hypertrophy in adult rats that had experienced a similar degree of chronic hypoxic hypoxia in early life along with persistent elevation of pulmonary artery pressure and pulmonary vascular resistance. It is unlikely that the left ventricular hypertrophy we have observed can be attributed to a persistent increase in systemic afterload in the neonatally hypoxic adult rats since our previous work has shown that systemic arterial pressure at rest [3] as well as during exercise [22] is not altered in adult rats that have been made hypoxic neonatally. Of interest is that left ventricular hypertrophy has been shown to accompany right ventricular pressure overload in both humans [23] as well as in animal studies [24].

Large human population studies suggest that abnormal conditions in fetal and infant life may cause various chronic diseases in later life as a consequence of altered physiologic or metabolic investigations at critical periods of early development [25]. Our findings may represent an example of abnormal physiologic programming resulting from hypoxemia in early life leading to long-lasting changes in myocardial adrenergic signal transduction. Although the mechanisms involved in such abnormal physiologic programming are not known, some speculation can be made regarding our results. The decrease in left ventricular adenylyl cyclase content may be the result of the interference of hypoxia-induced transcription factors on normal cardiac development. It is becoming increasingly clear that cardiac morphogenesis and development is the result of a complex spatio-temporal interaction of transcription factors, cardiac genes and cardiac cells [26]. Reduced oxygen is known to activate transcription factors such as HIF-1 and NF-kB which have wide ranging effects on gene expression [27]. Of interest is that both of these factors have been implicated in the expression of genes involved in cardiovascular differentiation [28]. Alternatively abnormal β-adrenergic stimulation during the period of neonatal hypoxia may have led to long-lasting changes in left ventricular adenylyl cyclase content. In the adult heart, prolonged β-adrenergic agonist stimulation leads to β-adrenergic receptor desensitization and down-regulation, while β-adrenergic receptor blockade or cardiac denervation leads to super-sensitivity of receptor signalling and ultimately to upregulation of cell surface β-adrenergic receptor number [4]. The situation is quite different in the neonatal heart. Neonatal β-adrenergic receptor stimulation results in sensitization of β-adrenergic receptor signalling as a result of changes down-stream of the receptors themselves including induction of adenylyl cyclase [29]. It has been suggested that the level of neural input during early life may fix the set-point for reactivity to future stimuli thus programming the responsiveness of the cell to its environment [29]. Of interest is that neonatal hypoxia in the rat produces long-lasting decreases in norepinephrine content and turnover in sympathetic ganglia, including the stellate ganglia, as well as in the heart [30] suggesting an impairment of functional cardiac sympathetic innervation. Such interference by neonatal hypoxia with the normal development of cardiac innervation could have prevented establishment of normal levels of adenylyl cyclase in our animals.

Our results show that the decreased left ventricular responsiveness to β-adrenergic stimulation in the adult following transient neonatal hypoxia is associated with markedly decreased left ventricular adenylyl cyclase activity and levels. While we suggest that it is likely that neonatal hypoxia causes decreased left ventricular responsiveness to β-adrenergic stimulation in adulthood primarily as a result of decreased left ventricular adenylyl cyclase levels, there are limitations to such a conclusion. We cannot exclude the possibility of additional long-term effects of neonatal hypoxia downstream of adenylyl cyclase. For instance Browne et al. [31] have shown that chronic hypoxia in fetal sheep results in a decrease in the ventricular inotropic response to extracellular calcium apparently due to decreased myofibrillar magnesium activated adenosine triphosphatase activity [32] as well as increased calcium binding to troponin C [33]. However, such effects have not been demonstrated postnatally nor have they been shown to persist to maturity. The decrease in adenylyl cyclase levels does not appear to have been due to a significant upregulation of G protein-coupled receptor kinase (GRK) activity. However, we did not systematically study alterations in the subcellular localization and/or activity of GRK2 or other ubiquitous or cardiac-specific GRK isoforms which may have been altered due to neonatal hypoxia. While we tried to minimize nutritional deprivation during the period of neonatal hypoxia by decreasing the litter sizes of the neonatally hypoxic rats, we
also cannot exclude the possibility that relative nutritional deprivation neonatally rather than hypoxia led to our observations. However, Pelouch et al. [34] were unable to demonstrate any effects of neonatal nutritional deprivation in the rat on the cardiac response to inotropic stimulation with isoproterenol at maturity. There is also a possibility that chronic pulmonary hypertension secondary to neonatal hypoxia produced our findings. Chronic right ventricular pressure overload has been shown to influence left ventricular function both in animals and humans apparently as a result of right ventricular influences on left ventricular geometry [35–38]. However, we have studied left ventricular function in an isolated preparation where the right ventricle was empty. Previous work on left ventricular myocytes from adult rats has also shown that the contractile response to isoproterenol, β-receptor density, isoproterenol induced cAMP increase and G protein-coupled receptor kinase activity are all unchanged by monocrotaline-induced pulmonary hypertension [39,40]. Finally we cannot exclude the possibility that the mild left ventricular hypertrophy we observed in neonatally hypoxic adult rats rather than the neonatal hypoxia itself led to our results. Various forms of experimental left ventricular hypertrophy are associated with decreased isoproterenol stimulated cAMP production [4]. However, in contrast to our results this appears to be due to post-transcriptional adenylyl cyclase regulation rather than decreased adenylyl cyclase transcription [41].

In conclusion, an extended period of neonatal hypoxia in rats has long-lasting effects on left ventricular adrenergic signal transduction at maturity. We suggest that the impaired effectiveness of adrenergic stimulation of the left ventricle is principally the result of decreased levels of myocardial adenylyl cyclase (type V/VI). Whether our findings are of clinical relevance must await human investigations. A long-lasting decrease in β-adrenergic responsiveness of the left ventricle could prevent individuals hypoxic in early life from producing rapid increases in ventricular function. Impaired β-adrenergic responsiveness might also become important after cardiac surgery if inotropic support becomes necessary.

Acknowledgements

This work was supported by operating grants from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council. CVR is a Chercheur Clinicien of the Fonds de la recherche en santé du Québec. TEH is a McDonald Scholar of the Heart and Stroke Foundation of Canada.

References

[23] Visner MS, Arentzen CE, Crumbley AJ, Larson EV, O’Connor MJ, Anderson RW. The effects of pressure-induced right ventricular...


