Long-term cardiovascular adaptations to neonatal hypoxia

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Abstract

INTRODUCTION: Previous work from the Rohlicek laboratory has shown that neonatal hypoxia is associated with an increase in systolic blood pressure in two month old male rats. We have asked here whether this increase persists into later maturity, and if it is also present in females. We have further examined separately whether sex hormones or alterations in autonomic control are implicated in this increase.

METHODS: Experiments were conducted on adult Sprague Dawley rats of both sexes. An experimental group was raised in hypoxia (FiO2 = 0.12) for the first ten days of life and subsequently raised in normoxia. A second group was reared entirely in normoxia. A subset of males and females were gonadectomized one month prior to recording. At two, three, and six months the rats were instrumented with an intravascular telemetric blood pressure transmitter monitoring abdominal aortic pressure. One week later arterial pressure was recorded for 24 hours in the ambulatory, unrestrained rats.

RESULTS: Systolic pressure was significantly higher in neonatally hypoxic male rats at every age during their active (night-time) period and as well at 3 and 6 months during the resting (daytime) period compared to controls. The effect size of neonatal hypoxia increased with age, although this increase did not achieve significance. Neonatally hypoxic females did not show any differences in systemic pressure compared to controls. Castration did not prevent the development of elevated blood pressure in two month neonatally hypoxic males, nor did ovariectomy unveil any differences between neonatally hypoxic and control females at three months of age. In two month male rats hypoxic neonatally, baroreflex sensitivity was significantly decreased during their active (night-time) period.

CONCLUSIONS: Our results indicate that the increase in blood pressure experienced by neonatally hypoxic adult male rats persists into later maturity. This effect appears to be sex specific to male animals. The finding of decreased baroreflex sensitivity following neonatal hypoxia at two months indicates that altered autonomic tone with a relative increase in sympathetic activity plays a role in the increase in arterial pressure.

RÉSUMÉ

INTRODUCTION: Des travaux antérieurs entrepris au laboratoire Rohlicek ont montré que l'hypoxie néonatale est associée à une élévation de la pression artérielle systolique chez les rats mâles âgés de deux mois. Dans le cadre de la présente étude, on demande si cette élévation persiste plus tard dans la maturité et si elle est également présente chez les femelles. On essaye en outre de déterminer si les hormones sexuelles ou des altérations dans le contrôle autonome jouent un rôle dans cette élévation.

MÉTHODE: Des études ont été menées sur des rats adultes Sprague-Dawley des deux sexes. Un groupe expérimental a été élevé en hypoxie ($FiO_2 = 0,12$) durant les dix premiers jours de vie et subséquemment en normaxie. Un second groupe a été élevé entièrement en normaxie. Un sous-ensemble de mâles et de femelles ont été gonadectomisés un mois avant la prise de mesures. À deux, trois et six mois, des rats étaient branchés à un transducteur de pression artérielle intravasculaire avec télémétrie pour surveillance de la pression de l'aorte abdominale. Une semaine plus tard, la pression artérielle a été mesurée durant 24 heures chez des rats ambulatoires et non contenus.

RÉSULTATS: La pression systolique a été considérablement plus élevée chez des rats mâles en hypoxie à tout âge durant leur période active (nocturne) et également à 3 et 6 mois durant la période de repos (diurne) par comparaison aux rats du groupe témoin. L'ampleur de l'effet de l'hypoxie néonatale s'est accrue avec l'âge, bien que cette augmentation n'ait pas été statistiquement significative. Les femelles en hypoxie néonatale n'ont montré aucune différence dans la pression artérielle générale par comparaison aux femelles du groupe témoin. Tout comme la castration n'a pu empêcher l'apparition d'une pression artérielle élevée chez les mâles en hypoxie néonatale âgés de deux mois, l'ovariectomie de même n'a pu montrer une quelconque différence entre les femelles en hypoxie néonatale par opposition aux femelles du groupe témoin à l'âge de trois mois. Chez les rats mâles âgés de deux mois en hypoxie néonatale, la sensibilité du baroréflexe a été considérablement atrophiée durant leur période active (nocturne).

CONCLUSION: Nos résultats indiquent que l'élévation de la pression artérielle chez les rats mâles adultes en hypoxie néonatale persiste plus tard dans la maturité. Cet effet semble être spécifique selon le sexe chez les animaux mâles. La découverte de la sensibilité du baroréflexe atrophiée à la suite de l'hypoxie néonatale à deux mois indique que le tonus autonome altéré, conjugué à une augmentation relative de l'activité sympathique, jouent un rôle dans l'élévation de la pression artérielle.

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PREAMBLE

The developmental origins of many adulthood diseases have been linked to adverse conditions experienced in early life. The periods before and after birth are of particular importance to the developmentally labile fetus or newborn, who are easily influenced by their external environments. This may be regarded as advantageous, whereby the ability to adapt in early-life may increase the probability of immediate-survival²; however, the consequences of an adapted physiology in early life may ultimately prove to be detrimental in adulthood. In this regard, a number of adult-onset diseases including cardiovascular disease, hypertension, and metabolic syndrome have been linked both experimentally and epidemiologically to a variety of prenatal and postnatal stresses²⁻⁵. For infants born with cyanotic congenital heart disease, hypoxemia is a clinically relevant postnatal stress that is experienced until surgical repair. This same population is at a significantly higher-risk of cardiovascular related deaths in adulthood despite surgical correction in infancy⁶. Emerging research suggests that hypoxemia in early life may be one factor contributing to the observed increase in late-hazard risk.

Our laboratory has previously shown that hypoxia in early life is associated with significant and diverse changes to the heart and vasculature at maturity^{5, 7, 8}. Recently, we have observed an increase in systolic blood pressure in two month old male rats⁵, a condition that in humans is known to increase both morbidity and mortality⁹. The decrease in arterial compliance after neonatal hypoxia⁵ and the association between chronic sympathoexcitation and hypertension¹⁰ strongly suggest an alteration in autonomic cardiovascular control, likely involving the baroreflex. Additionally, both human and animal studies indicate gender related differences in systemic arterial pressure that become apparent after sexual maturity¹¹. Sex differences are also apparent in animal models of arterial blood pressure programming by early life stress^{12, 13}. In this regard, two separate avenues have been pursued. The first explores the development of systemic arterial pressure from early to later maturity in both male and female rats following neonatal hypoxia and the potential involvement of sex hormones. The second sets out to determine if altered autonomic cardiovascular control is implicated in the increase in systemic arterial pressure described by Ross⁵ by examining baroreflex sensitivity.

This thesis begins with a summary of several fundamental aspects of the cardiovascular system and how they are influenced by the autonomic, renal, and renin-angiotensin systems. The objective of the Introduction is to allow the reader to contextualize the results in regards to the potential mechanisms which have been proposed in the Discussion. It further highlights the significance of elevated blood pressure, the developmental origins of adulthood disease, and the deleterious effects of neonatal hypoxia that are relevant for adult 'survivors' of cyanotic congenital heart disease.

List of Commonly Used Abbreviations

acetylcholine (ACh); angiotensin-II (Ang-II); angiotensin converting enzyme (ACE); castrated (CAS) ; caudal ventrolateral medulla (CVLM); confidence interval (CI); epinephrine (EPI); G protein-coupled receptor (GPCR); gammaamino butyric acid (GABA); heart rate variability (HRV); intrauterine growth restricted (IUGR); norepinephrine (NE); nucleus tractus solitaries (NTS); ovariectomized (OVX); pulse interval (PI); renin-angiontensin system (RAS); rostral venterlateral medulla (RVLM); sex steroid hormones (SSHs); spontaneously hypertensive rats (SHRs); standard error of the mean (S.E.M.); systolic blood pressure (SBP)

INTRODUCTION

The cardiovascular system transports nutrients, gasses, hormones, waste, and heat to meet the various metabolic and physiologic demands of the body. It is composed of a network of transport and exchange vessels (the systemic and pulmonary circulations), a pump (the heart), and a medium for transportation (the blood). Regulatory mechanisms, as well as plasticity of growth during development, can and often do alter the mechanical and/or physical properties of one or more aforementioned arms of the cardiovascular system. These adaptations, which can be acute or long-lasting, occur in different physiologic and pathologic situations including (but not limited to) perinatal stresses, exercise, digestion, sleep, oxygen deprivation, mental stress, and numerous adverse disease states. To better understand the mechanisms and consequences of cardiovascularrelated diseases a basic overview of the relevant anatomy and principles of the cardiovascular system and its regulatory components are presented.

The Heart

A vast and complex network of genes and molecular pathways initiate and continue the development of the human heart beginning at day 15 to 16 of gestation¹⁴. The rapid proliferation of cardiomyocytes (hyperplasia) only continues until late gestation, at which point adaptation to increased hemodynamic load and changes in fetal cardiac gene expression results in increased cell size (hypertrophy)¹⁵. The timing of the switch from proliferation to hypertrophy is not conserved across species, and occurs between postnatal days 4 to 5 in rodents¹⁶. Normal development results in a four-chamber organ that is asymmetrically divided into left and right sides, each containing a superior atrium and inferior ventricle. Separating the superior atriums from the inferior ventricles is a plane of connective fibrous tissue. This barrier prevents electrical conduction within the atria from immediately passing to the ventricles, and also contains four unidirectional valves that permit blood to pass from each atrium into its respective

ventricle and from each ventricle into either the pulmonary artery or the aorta. The ventricles are the major pumping chambers of the heart and so develop a relatively larger muscle mass than the atria. The left ventricle has a significantly higher hemodynamic load than the right ventricle, resulting in a thicker muscular wall as well.

The propagation of electrical activity in the 'normal' heart that leads to atrial and ventricular contraction follows the same path with every heartbeat. It is initiated by a band of spontaneously depolarizing pacemaker cells, collectively called the sinoatrial node, which is located along the junction of the superior vena cava to the right atrium. Propagation of the electrical wave across the atria is delayed at the slow-conducting atrioventricular node, giving enough time for the atria to relax before ventricular contraction occurs. The atrioventricular node is connected to a group of fast conducting cells (the bundle of His) that travels through the fibrous tissue defining the atrioventricular boundary. The bundle of His further branches into the left and right ventricles to very quickly initiate coordinated ventricular contraction. Although there are several regions in the heart with pacemaker activity, the sinoatrial node normally fires the fastest and therefore determines heart rate. At the cellular level heart rate, as well as several other processes that lead to contraction, is a reflection of the state of the numerous ionic channels that change membrane potential and intracellular ion concentrations. The state of each ion channel is strongly influenced by adrenergic and cholinergic stimulation.

Classically, the sequence of contractions within the cardiac cycle is described in terms of the volume and pressure found within the left ventricle and consists of ventricular systole and ventricular diastole. From this perspective, ventricular systole beings with isovolumetric contraction of the left ventricle. As left ventricular pressure rises above that found in the left atrium the left atrioventricular valve, or the mitral valve, closes. Once pressure exceeds that of the systemic circulation the second valve of the left ventricle, the aortic valve, opens and blood is ejected into the major conductance artery of the systemic circulation, the aorta. As pressure in the left ventricle drops below that of the aorta the aortic valve closes and so begins the second phase of the cardiac cycle, ventricular diastole. Isovolumetric relaxation of the left ventricle continues until pressure drops below that found in the left atrium and mitral valve opens. At first, blood flows passively down a pressure gradient until the atria actively contract and supply an extra push of blood into the ventricles. This marks the end of ventricular diastole and with contraction immediately following, the return of ventricular systole¹⁷.

The heart is richly innervated with receptors that respond to neurohumoral signalling. Central connections from the cardiovascular center of the medulla oblongata to the heart are made by both sympathetic and parasympathetic efferents¹⁸. These release norepinephrine (NE) and acetylcholine (ACh), respectively. The heart also responds to circulating epinephrine (EPI) and (to a lesser extent) NE secreted from the adrenal medulla. Both the adrenergic agonists NE and EPI and the cholinergic agonist ACh signal through the G Protein-Coupled Receptor (GPCR) family. The hallmarks of this family of receptors originate from their seven transmembrane α -helices and their interaction with heterotrimeric G proteins. Initiation of the signalling sequence is mediated by a trimer of G-proteins containing one subunit from each of the three protein families: G_{α}, G_{β} , and G_{γ} . When a ligand binds to the GPCR, GDP on the G_{α} subunit is exchanged for GTP, allowing for the disassociation of the trimer into a G_{α} monomer as well as a G_{β} and G_{γ} dimer. These two separate entities can then go on to initiate a variety of downstream effects, many of which are mediated by second-messengers¹⁷. Several isomers of the G_{α} subunit exist that are specific to various receptors and which activate different downstream pathways. The adrenergic agonists NE and EPI signal through α_1 , α_2 , β_1 , and β_2 adrenergic GPCRs, while the cholinergic agonist ACh signals through muscarinic M₂ GPCRs. Cardiac adrenergic receptors are mostly β_1 and have a greater affinity for NE than EPI. Although ubiquitous in the heart, these receptors are found in much higher densities in the sinoatrial node and ventricular myocardium than

elsewhere⁹. M_2 receptors are also located throughout the heart, and despite the ventricular myocardium having a similar receptor density than the atrial myocardium, vagal innervations to ventricular myocardium are reduced^{9, 19}.

The Circulation

The circulatory system is separated in two by the right and left sides of the heart. Together the pulmonary and systemic circulations form a closed loop with a relatively fixed volume of blood, although effectively this can be increased or decreased in a variety of ways that will be presented later. In both cases blood leaves the heart through relatively thick arteries that eventually divide into very thin-walled capillaries. These coalesce into veins that return blood to the heart. The pulmonary circulation flows between the right heart and left heart while the systemic circulation, the focus of this study, flows from the left heart to the right heart. More specifically, deoxygenated blood returning from the vena cava is collected in the right atrium and passes through the tricuspid valve into the right ventricle. The deoxygenated blood is pumped from the right ventricle through the pulmonary valve where it enters the pulmonary circulation via the pulmonary artery. As the pulmonary artery approaches the lungs it divides into smaller sections until the vessels are the size of capillaries, allowing for the diffusion of gases across the alveoli of the lungs. Oxygenated blood returning from the pulmonary vein arrives in the left atrium and is sent into the left ventricle through the mitral valve, where it is pumped into the systemic circulation via the aortic valve and aorta.

The arterial system, of which this study is primarily concerned, is structurally different from the venous system. The large diameter aorta is predominantly composed of elastic tissue with some smooth muscle and fibrous tissue. This composition provides structural integrity and allows for the distension and absorption of potential energy during ventricular systole²⁰. During diastole this elastic potential energy is released and provides a moving force in the absence of ventricular contraction. As the aorta branches into the periphery, pressure drops

along with arterial diameter while the relative amount of smooth muscle increases until it reaches a maximum at the arterioles²⁰. The abundance of smooth muscle helps dampen the pulsatile pressure wave created by the phasic contraction cycle and is the major determinant of systemic vascular resistance²⁰. Control of smooth muscle tone at this level also allows for precise regulation of local blood flow. The arterioles branch into capillaries, which have a single-cell epithelial layer surrounded by a basement membrane. The thin walls allow for very efficient diffusion of gases and nutrients. Capillaries coalesce into venules and veins of increasing diameter. The venous system is a low-pressure, high-capacitance system that accommodates the transport of large volumes of blood back to the right atrium²⁰. Two-thirds of blood volume is stored here, and smooth muscle control over venous diameter is an important blood pressure regulatory mechanism.

The circulatory system responds to central, autonomic, local metabolic, and mechanical controls. In contrast to the heart, the circulation is primarily innervated by sympathetic efferents that release NE and act on α_1 - and (to a lesser extent) α_2 - and β_2 -adrenergic receptors^{18, 19}. Circulating EPI is highly selective for β_2 -adrenergic receptors. This changes at high physiological concentrations, where β_2 - selectivity for EPI is equal to that of α_1 - receptors. The parasympathetic system innervates a few select regions, including the blood vessels of the head and viscera, genitalia, bladder, and large bowel. Its effect on peripheral resistance is therefore negligible²⁰. The vasculature also contains the AT-2 receptor, which is a GPCR that responds to circulating Angiotensin-II (Ang-II) originating from the kidneys⁹. In addition to the structural features of the vasculature that enable extrinsic control, the inner endothelial lining of vessels respond to the sheer stress of blood flow or to endothelial damage, releasing nitric oxide (NO) or endothelin-1, respectively, in the process⁹.

Blood Pressure

Ultimately, the heart and circulation work in unison to control blood pressure and perfusion to meet the metabolic needs of individual organs, tissues, and cells. Measurements of blood pressure often quantify the pressure in the large arteries (the brachial artery is commonly used as an approximation), with key variables noted for the maximal (systolic blood pressure), minimal (diastolic blood pressure), or mean arterial pressure (calculated from the area under the blood pressure curve, or roughly diastolic $+\frac{1}{3}$ (systolic-diastolic), due to the prolonged time spent in ventricular diastole). Pulse pressure is simply the difference between the systolic and diastolic blood pressure values. These variables carry both physiological and clinical significance, and their meanings will be explored in the proceeding sections.

The calculation of arterial pressure relies on the same mathematical principles that are used to quantify pressure in any other system. That is, mean arterial pressure is equal to the product of flow and resistance. Flow in this case is determined by the cardiac output, which itself is the product of stroke volume and heart rate, whereas resistance is largely determined by the peripheral vasculature¹⁸. These physiological factors are in turn modified by several physical factors that influence not only the aforementioned variables but also the shape of the pressure waveform. Namely, the volume of fluid and the compliance of the arteries²⁰. Strictly speaking, the volume in the arterial system at any one point is the difference between the input (cardiac output) and the output (peripheral runoff). Compliance quantifies the relationship between changes in pressure and volume within a given system ($C = \Delta Volume / \Delta Pressure$). Arterial compliance often refers to the large arteries, which distend and absorb most of the potential energy over the course of a cardiac cycle. Variations around mean arterial pressure exist because the volume of blood leaving the periphery during ventricular diastole occurs at a slower rate than the volume of blood entering the arteries during ventricular systole (equal to the stroke volume)²⁰. This increase in volume is initially accommodated by the distension of the aorta. The more compliant the aorta, the more volume it can accommodate to dampen the peak in the pressure wave. Therefore systolic blood pressure, the peak of the pressure wave, is largely determined by the compliance of the large arteries. Systemic vascular resistance on the other hand is responsible for the volume of blood leaving the arterial system, and therefore determines the pressure achieved at the lowest point of the pressure waveform (diastolic blood pressure). It is important to note that mean arterial pressure is not directly influenced by compliance, only the rate at which a new average pressure is achieved²⁰.

Mechanisms of Blood Pressure Control

There are many opposing, redundant, and interdependent pathways that regulate systemic and regional blood pressures. Those mechanisms that regulate regional pressures generally do not alter mean arterial pressure, and have been selectively omitted based on relevance. This section gives a brief background on short-term autonomic control of blood pressure, its regulation by the baroreflex, and long-term control via the renal and renin-angiotensin systems. Focus is given to those mechanisms that have been found to be involved in the (programmed) development of hypertension and cardiovascular disease.

Short-term Control: The Autonomic System

As was previously stated, any alterations to stroke volume, heart rate, cardiac output, systemic vascular resistance, compliance, or blood volume will have an effect on the blood pressure waveform (provided changes to cardiac output and systemic vascular resistance are not opposite and proportional). Activation of the autonomic system is pervasive and has the ability to regulate all of the aforementioned variables to achieve an effective and coordinated physiological response. The sympathetic and parasympathetic branches of the autonomic system normally oppose each other and are generally reciprocal. Their activity levels change with the onset of exercise, mental or emotional stress,

excitation, the time of day, or input from sensory receptors like the chemo or baroreceptors⁹.

The sympathetic efferents that innervate the heart and vasculature release the catecholamine NE. Binding of NE (and some circulating EPI released from the adrenal medulla upon sympathetic stimulation) on cardiac β_1 -adrenergic receptors activates the $G_{\alpha s}$ subunit in the sinoatrial and atrioventricular nodes and ventricular myocardium. $G_{\alpha s}$ activates adenylyl cyclase which converts adenosine triphosphate (ATP) into cyclic AMP (cAMP). The latter activates the third messenger protein kinase A (PKA), which through phosphorylation regulates intracellular Ca²⁺ levels in three ways. The first promotes the influx of extracellular Ca²⁺ into the cytosol through phosphorylation of plasma-membrane L-type Ca²⁺ channels. Second, phosphorylation of ryanodine receptors result in the release Ca²⁺ by the sarcoplastic reticulum. Finally, PKA releases inhibition of phospholamban on the sacroendoplasmic reticulum calcium pump (SERCA 2a in the heart)¹⁷. The former two increase contractility (inotropy) in the myocardium, while the latter along with PKA phosphorylation of troponin-I (which decreases calcium sensitivity of troponin C and therefore increases the rate of crossbridge detachment) increase the relaxation rate (lusitropy)^{9, 17}. In the sinoatrial node heart rate is accelerated (chronotropy) when cAMP directly increases the depolarizing funny current (inward Na⁺), and indirectly (via PKA) increases the depolarizing L- (long-lasting) and T- (transient) type Ca^{2+} channels, and the inward reploarizing K^+ current¹⁷. Conduction is also accelerated (dromotropy), especially within the slowly-conducting atrioventricular node, by increasing the depolarizing slope associated with the L-type Ca²⁺ channel¹⁹. NE also acts upon a small amount of cardiac α_1 -adrenergic receptors to mildly increase inotropy through phospholipase C (α_1 - signalling detailed below). To summarize, sympathetic activation of the heart results in an increased inotropic, lusitropic, chronotropic, and dromotropic response. Mean arterial pressure is therefore increased through an accelerated heart rate and elevated stroke volume.

The response of the vasculature to sympathetic stimulation is largely mediated by α_1 -adrenergic binding of NE. $G_{\alpha q}$ subsequently activates phospholipase C (PLC), which hydrolyzes membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP₂) to generate the second messengers inositol triphosphate (IP_3) and diacylglycerol $(DAG)^{17}$. The final result is an increase in smooth muscle contraction mediated by the release of internal Ca^{2+} through IP₃-gated channels on the sarcoplastic reticulum, and activation of the proliferative signalling protein kinase C (PKC) by DAG^{9} . These pathways contribute to the basal level of vascular tone. Thus, vascular dilation can also be achieved through reduced sympathetic activity¹⁸. Sympathetically induced release of EPI may result in either smooth relaxation or contraction, depending on its concentration and therefore receptor affinity. At low concentrations, β_2 -adrenergic receptor activation stimulates the same $G_{\alpha s}$ pathway as cardiac β_1 - receptors do; however, whereas in cardiac muscle PKA increases intracellular Ca²⁺ which promotes contraction via troponin-C, in vascular smooth muscle Ca^{2+} binds to calmodulin which promotes relaxation via inhibition of myosin light chain kinase¹⁹. At high concentrations of EPI, α_1 - selectivity is equal to that of β_2 - and contraction prevails. By far the most common result of sympathetic activation on the vasculature is smooth muscle contraction of the arterioles and the venous capacitance system that results in an increase in systemic vascular resistance and venous return. The latter increases mean arterial pressure through the Frank-Starling mechanism, which states that increasing the end-diastolic volume (preload) optimizes the cardiomyocyte sarcomere length-tension relationship to increase stroke volume¹⁸.

The parasympathetic system generally regulates mean arterial pressure through control of heart rate. Complete autonomic blockade reveals an intrinsic firing rate of 100 beats/min in the human sinoatrial node, which under normal conditions of predominantly parasympathetic control fires closer to 60 beats/min¹⁷. This negative chronotropic effect begins when ACh binds to the cardiac M₂ receptor within the sinoatrial node. First, the $G_{\alpha i}$ monomer inhibits adenylyl cyclase, resulting in decreased cAMP and ultimately inhibiting the depolarizing funny (Na⁺) and Ca²⁺ currents. Secondly, the $G_{\beta\gamma}$ dimer directly opens the inward rectifier potassium current¹⁷. In the atrioventricular node, the $G_{\alpha i}$ subunit's inhibition of adenylyl cyclase decreases conduction velocity while in the atrium (and to an even lesser extent in the ventricles) it results in decreased myocardial contractility. The parasympathetic system additionally inhibits sympathetic NE release through presynaptic M₂ receptors located on the terminal varicosities of sympathetic efferents⁹. Thus, the cardiac response to parasympathetic stimulation can be viewed as antagonistic to the sympathetic pathway, and is summarized by decreased chronotropy, dromotropy, and inotropy that all contribute to lower heart rate, stroke volume, and ultimately mean arterial pressure.

Regulation of the Autonomic System: The Baroreflex

Although several of the major mechanisms controlling blood pressure are evidently influenced by the nervous system, those regulating autonomic neural output have not been covered. In this regard, the arterial baroreflex can be viewed as one of the most important determinants of blood pressure because of its pervasive control of both the sympathetic and parasympathetic systems. From a functional point of view, this primarily reduces short-term fluctuations in blood pressure (e.g. spontaneous and orthostatic changes). Baroreceptor stimulation (an increase in arterial pressure) results in an acute inhibition of sympathetic output to the heart and vasculature and an increase in vagal output to the heart. The net effects are a decrease in heart rate, venous return (*i.e.* stroke volume), systemic vascular resistance, and cardiac output that decreases mean arterial pressure back to a hypothetical set-point²¹. Conversely, decreasing arterial pressure releases sympathetic inhibition and reduces vagal outflow. This implies the presence of a set-point, above and below which the baroreflex alters its tonic level of activity. What defines the set-point is complicated by the presence of two types of baroreceptors, which respond to the mean pressure and the fluctuations in it (i.e. mean arterial and pulse pressures). Additionally, they may adapt to persistent changes in pressure²². Long-term regulation of arterial blood pressure by the baroreflex, although controversial, is believed to involve the set-point around which activation occurs and regulation of the sympathetic efferent branch, which additionally contributes to renal sympathetic nerve activity²². A more complete description of the baroreflex requires knowledge of the level of efferent output in relation to afferent input. Thus, baroreflex sensitivity commonly quantifies changes in heart rate in response to changes in arterial pressure (note that the input and output variables can vary depending on the specific part of the long baroreflex pathway being studied).

The terminal sensory afferents of the arterial baroreflex are located within the adventitia of the carotid sinuses and transverse section of the aortic arch. Baroreceptors respond to arterial distension (and therefore arterial pressure, although indirectly) and mechanically tranduce this information through the opening of depolarizing cation channels²¹. These mechanotransducers are tonically active under normal physiological conditions and are sensitive to both mean arterial and pulse pressures²⁰. Afferent information travels from the aortic and carotid baroreceptors to the nucleus tractus solitaries (NTS) via the vagus and glossopharyngeal nerves, respectively. Baroreceptor afferents activate excitatory glutamatergic receptors in the NTS that activate both sympathoinhibitory and parasympathoexcitatory pathways originating in the caudal ventrolateral medulla (CVLM). The parasympathetic branch stimulates preganglionic parasympathetic neurons in the ventrolateral nucleus ambiguus, initiating reflexive bradycardia via cardiac ganglionic neurons²¹. The sympathetic pathway releases the inhibitory neurotransmitter gamma-amino butyric acid (GABA) on pre-sympathetic motor neurons in the rostral venterlateral medulla (RVLM) arresting transmission to postganglionic sympathetic neurons most notably innervating the heart, vasculature, and kidneys 23 .

The RVLM sympathetic efferents are constantly modified by nonbaroreflex factors, including central and peripheral chemoreflexes, lung stretch receptors, muscle metabotropic receptors, visceral and cutaneous noiceptors, mental stress, and several disease states^{21, 23}. Input to the RVLM typically results in a homogenous inhibitory or excitatory response; however, absolute activity levels of the organotopically organised efferents may be differentially regulated (*e.g.* preferential activation of renal sympathetic efferents)²³. The NTS also receives non-baroreflex input from similar sources as well as the hypothalamus and circulating hormones such as Ang-II. This latter hormone promotes the release of NO from the endothelium, which then increases GABA release in the NTS²³. Together, regulation of the NTS, CVLM, and RVLM and their efferent projections can change both BRS and the set-point around which the baroreflex operates.

Long-term control: The Renal and Renin-Angiotensin Systems

Although the kidneys are generally not considered to be a fundamental part of the cardiovascular system, they play an integral role in the maintenance of blood volume/pressure. This is mainly achieved through the regulation of plasma osmolarity (Na⁺ salts) and water balance. Control over the normal renal mechanisms that accomplish this is mediated by several major hormones and reflexes, the most relevant of which will be reviewed. A more detailed examination of renal structure and function can be found elsewhere²⁴.

Renal blood flow and the glomerular filtration rate (GFR) are controlled by changing the resistance of the interlobular arteries as well as the afferent and efferent arterioles that supply and drain the glomerulus. Their smooth muscle (especially in the afferent arteriole) has α_1 -adrenergic receptors that increase resistance and decrease blood flow to the nephrons²⁴. Ligands include both NE and dopamine directly released by sympathetic efferents and indirectly circulating EPI released from the adrenal medulla. Several other hormones also control vascular tone within the kidneys. The most significant of these is angiotensin-II (Ang-II), a potent vasoconstrictor that has diverse and far-reaching effects on many aspects of renal function and blood pressure. This hormone is part of the renin-angiontensin system (RAS) that is stimulated by several factors including the sympathetic system. After the ultrafiltrate is produced, the process of reabsorption within the distinct sections of the nephron becomes the most critical aspect determining final urine osmolarity and volume. The renal sympathetic nerves participate in this through their innervations of the adrenal medulla, posterior pituitary gland, juxtaglomerular (JG) apparatus, proximal tubule, loop of Henle, distal tubule, and collecting $duct^{24}$. Once again, activation releases locally produced and circulating catecholamines that alternatively increases Na⁺ (and therefore water) reabsorption into the interstitial space surrounding the latter four sections of the nephron. Sympathetic activation also promotes the release of antidiuretic hormone (ADH) from the posterior lobe of the pituitary gland, which is a potent regulator of water balance. Its main actions are mediated by increasing the number of aquaporin-2 channels on the collecting duct and stimulating thirst centers located in the hypothalamus. Minor, secondary effects of ADH include increased Na⁺ reabsorption from the thick ascending limb of the loop of Henle, the distal tubule, and the collecting duct (from which urea reabsorption is also increased and results in greater osmolarity of the interstitial space)²⁴. Aldosterone selectively increases Na⁺ reabsorption by the same means and to a much greater degree than ADH. This steroid hormone is produced in the adrenal medulla and is secreted in response to Ang-II signalling²⁴.

The RAS and sympathetic system are critically linked and have synergistic effects on the cardiovascular system. The RAS participates in the maintenance of blood pressure homeostasis most notably by increasing vascular tone, sympathetic activity, cellular growth, and aldosterone release⁹. This response is mediated by Ang-II, which is the final, active cleavage product of the RAS. Ang-II synthesis begins when angiontensinogen produced by the liver is cleaved into angiotensin-I by renin. Angiotensin-I is further cleaved into the active hormone Ang-II by angiotensin converting enzyme (ACE) located in the endothelium of the vasculature (especially the lungs and coronary arteries). The limiting reagent in this process is renin, whose main source in the body is the JG cells of the kidney¹⁸. These cells, located within the endothelial wall of the afferent arteriole, are part of the JG apparatus that is also home to the adjacent macula densa cells found along the distal lumen of the thick ascending loop of Henle²⁵. Secretion of

renin from the JG cells is directly stimulated by cAMP (via β_1 -adrenergic receptors), decreased renal perfusion pressure, and NaCl delivery to the macula densa cells²⁴. β_1 - activation within the JG cells is slightly different than most smooth muscle. It is known to signal through the adenylyl cyclase V isoform that is inhibited by Ca^{2+} and is suggested to promote Ca^{2+} efflux²⁵. Thus, in contrast to virtually every other secretory cell, renin release is greater when intracellular Ca²⁺ concentrations are low and inhibited when high²⁵. Renin release eventually results in elevated Ang-II signalling via AT₁ receptors found throughout the vasculature and nervous system. Both α_1 -adrenergic and AT₁ receptors signal through the $G_{\alpha q}$ monomer. Accordingly, in the vasculature the AT₁ pathway results in a similar opening of IP_3 -mediated Ca^{2+} channels on the sarcoplastic reticulum and concomitant vasoconstriction⁹. In addition to having NE-like effects on the vasculature, Ang-II directly interacts with presynaptic AT₁ receptors to increase NE release and decrease NE reuptake, brainstem nuclei to increase sympathetic output, autonomic ganglia to facilitate neurotransmission, and endothelial cells to release the potent vasoconstrictor endothelin⁹. These are in addition to the aforementioned direct and indirect effects on renal perfusion and Na⁺ reabsorption.

Pathophysiology of Blood Pressure: Hypertension

The development of elevated blood pressure occurs both naturally with age (commonly manifested as isolated systolic hypertension) and as a consequence of genetic, environmental, and other risk factors (*i.e.* obesity, smoking, etc.)²⁶. Furthermore, differences in this pattern exist between the sexes¹¹. From as early as puberty and for the majority of life, men generally exhibit higher blood pressures than age-matched, premenopausal women^{27, 28}. Conversely, blood pressure in postmenopausal women increases to levels equal to or greater than males^{27, 29}. Normal levels of arterial pressure allow for sufficient perfusion of tissues while minimizing the damage associated with increased pressure. 'Normal' values for systolic and diastolic blood pressures are cited at 120 mmHg and 80 mmHg, respectively. The artificial cut-off that defines a

pathological elevation in blood pressure (hypertension) is currently 140/90 mmHg; and although this is useful for classifying those most at risk for developing associated morbidities, it is rather superficial considering the important linear association between elevated blood pressures (beginning as low as 115/75 mmHg) and mortality from stroke and coronary heart disease³⁰. Thus, when studying elevations in blood pressure, small increases may be highly consequential when applied to entire populations.

The underlying causes of hypertension very often influence several of the physiological determinants or control mechanisms of arterial pressure. Hypertension is not a direct cause of mortality per se, but over time asymptomatic, chronic elevations of blood pressure damage the structure and function of the vasculature, heart, and various organs. Clinically this is manifested as retinopathy, dementia and ischemia of the brain, microalbuminuria and glomerulopathy, coronary artery disease and left ventricular hypertrophy, and arteriosclerosis of the peripheral vasculature that ultimately leads to death²⁶. Considering the pervasive damage caused by hypertension, it is not surprising that it is the number one risk factor for mortality (responsible for 7.5 million or 13%) of annual deaths globally), and in 2000 was estimated to effect almost 1 billion, or a quarter of the world's adult population³¹. Conservative predictions estimate that almost a third of the world's adult population (1.56 billion) will be affected in 2025. This does not account for a shift in population demographics towards an increasing proportion of high-risk elderly individuals³¹. Therefore, interventions that reduce the burden of hypertension and that identify those most at risk are of great significance.

The Developmental Origins of Adulthood Disease Hypothesis (The Barker Hypothesis)

In 1986, Barker and colleagues published a seminal study on the link between adulthood disease and infant mortality³². They analyzed the population censuses of different boroughs in England and Wales for cause of death between

1968 and 1978 and for rates of infant mortality from 1921-1925. Rates of several adult causes of death, including ischemic heart disease, were positively associated with infant mortality rates from decades earlier. This finding led Barker and associates to hypothesize that stress associated with poor living conditions experienced during a critical fetal and neonatal development window may predispose an individual to cardiovascular diseases in adulthood. Shortly thereafter he refined his hypothesis, in particular the stress associated with poor living conditions, in a detailed retrospective study that found weight at birth and at one year strongly and inversely correlated with rates of deaths from ischemic heart disease in later life³³. Although several papers with very similar conclusions had been previously published³⁴⁻³⁶, Barker's findings pushed fetal and neonatal malnutrition into the spotlight, the subject of which has since developed into an extensive area of research. The "Barker hypothesis", or "developmental origins of adulthood disease hypothesis", has been used to explain the potential aetiologies of a number of adulthood ailments including, but not limited to, obesity, Type 2 diabetes, hypertension, and cardiovascular disease (reviewed extensively by McMillen et. al.²). A modern interpretation of the developmental origins (of adulthood disease) hypothesis suggests that various independent stressors, introduced at a critical period of ontogeny, may trigger an adaptive response that initially favours immediate survival. As the metabolic environment changes from one of stress and depravity to relative affluence later in life, these physiological adaptations may become maladaptive.

This contemporary view of how adulthood diseases can begin even before birth has developed from a deeper questioning of the causes and consequences of low birth weight. It is reasonable to claim that low birth weight is an indicator of fetal and neonatal stress, and is not a direct cause of adulthood disease. When viewed as a surrogate measure of fetal growth and a general indicator of overall wellbeing, the conclusions drawn still remain contentious in that confounding factors and causal relationships are very often difficult to discern. Thus, controlled animal studies have allowed for a more precise examination of the initiating stressors (*e.g.* placental insufficiency, global or targeted maternal nutrient restriction, maternal exposure to glucocorticoids, hypoxia, etc.), the physiological 'adaptations' (*e.g.* decreased nephron or cardiomyocyte number, structural and/or functional changes of the vasculature, etc.), and the development of adulthood diseases that follow (*e.g.* hypertension, Type 2 diabetes, obesity, cardiovascular disease, etc.)². Interestingly, these adaptations appear to be influenced by sex. For example, several animal models suggest that the female sex is relatively protected from prenatal stresses that lead to hypertension¹³. Considering the enormous social and financial burdens of these diseases, there is great relevance in understanding the physiology behind specific patient populations that experience fetal and/or neonatal stress.

A Patient Model of the Developmental Origins Hypothesis: Cyanotic Congenital Heart Defects

Congenital heart defects are the most prevalent of birth defects, with the incidence ranging from 6-8 per 1000 live births³⁷. These can be further classified into non-cyanotic and cyanotic heart defects, with the latter occurring in approximately 1 in 1400 live births³⁸. Cyanosis (blue discolouration of tissue associated with deoxyhemoglobin) can result from various heart malformations that, for an array of reasons, do not allow for adequate delivery of blood to the lungs. The most common cyanotic congenital heart defect is Tetralogy of Fallot, which is characterized by four anatomical anomalies: 1) pulmonary outflow tract obstruction, 2) ventricular septal defect, 3) overriding aortic root, and 4) right ventricular hypertrophy³⁹. For the majority of modern medical history, these patients had next to no therapeutic options available; instead, the clinical course was more an exercise of prognosis rather than treatment. This changed in 1944 when, with the expert advice of Helen Taussig, Alfred Blaock performed the first systemic to pulmonary artery shunt (now referred to as the BT-shunt)^{39, 40}. This enabled the recirculation of desaturated systemic blood back through the pulmonary circulation, ultimately increasing pulmonary blood flow and thus systemic arterial saturation. The next few decades saw direct intracardiac repair of the atrial septal defect, to complete cardiac repair. Advances in surgical technique over this same period have drastically improved immediate survival rates, such that perioperative survival now approaches 98%³⁹. This has had important implications for congenital heart specialists, whose focus has shifted from improving immediate survival for a neonatal and infant population towards the long-term risks associated with an increasing (and now out-numbering) population of adult survivors³⁹. Interestingly, as the short-term hazard risk has improved from a 27% risk of mortality one year after repair in earlier surgical eras to approximately 2% in more recent ones, the late hazard risk has remained unchanged over time⁶. This implies that something independent of surgical era is conferring risk to adult survivors. One possibility is that chronic exposure to hypoxia in infancy before surgical repair has deleterious, long-term consequences on adult survivors of cyanotic congenital heart defects. Indeed, these 'survivors' are under constant threat of arrhythmias, ventricular dysfunction, re-operation, sudden cardiac death, myocardial infarction, and stroke⁴¹.

Hypoxia as a Neonatal Stress

As the field of developmental programming transitioned from epidemiological to laboratory studies, investigators gained control over the type, intensity, and timing of stress applied. Considering normal development of the fetal heart is dependent on the balanced induction of hypoxia dependent genes (*e.g.* hypoxia inducible factor 1 [HIF-1] and vascular endothelial growth factor [VGEF]), it is not surprising that too much or too little oxygen during the fetal period disrupts heart development and increases susceptibility to stress later in life (reviewed by Patterson⁴²). Moreover, the developmentally sensitive period that is responsive to hypoxia has been found to extend into postnatal life, and is not confined to the cardiovascular system. Newborn rats who were transiently exposed to chronic hypoxia (FiO₂ = 0.10, days 1-6 of life), subsequently reared in normoxia, and assessed in adulthood displayed an increase in minute ventilation, anteroposterior dimensions of the throax, lung mass, respiratory compliance, and diaphragmatic surface area^{43, 44}. Furthermore, they displayed a blunted ventilatory

response to acute hypoxia and a prolonged inspiratory time after end-expiratory occlusion (*i.e.* the Hering-Breuer reflex, an indicator of central respiratory control)⁴⁵. The same episode of chronic hypoxia in adulthood did not result in any associated structural or functional changes after return to hypoxia, further supporting the notion of a developmentally sensitive period unique to early life.

Similar experimental protocols, in particular those done by Rohlicek et. al., have demonstrated pervasive cardiovascular alterations after chronic hypoxia during the immediate postnatal period. In one study, isolated and perfused hearts of adult rats exposed to an F_iO_2 of 0.12 for days 1-10 of life were studied at increasing concentrations of dobutamine (a β_1 -receptor agonist). Although at low concentrations the maximal left ventricular pressure and rate of pressure generation was equivalent in neonatally hypoxic and control rats, increasing concentrations of dobutamine revealed a blunted inotropic response in the neonatally hypoxic group⁸. This was not linked to a difference in adrenergic receptor number, but to a decrease in the second messenger adenylyl cyclase V/VI in neonatally hypoxic rats. In addition to changes in protein expression, left ventricular gene expression was markedly altered immediately following and long after exposure to hypoxia in early life⁷. Gene chip and RT-PCR analyses revealed over 400 genes that were either upregulated or downregulated at 90 days of age in rats hypoxic neonatally compared to controls. These included genes involved in vascular remodelling, angiogenesis, cellular metabolism, energy homeostasis, and apoptosis. Thus, hypoxia in early postnatal life can have significant and lasting changes on left ventricular gene expression that continues to unfold well into maturity. To further examine the long-term effects of hypoxia in early-life, additional structural and functional tests were performed at 90 days⁴⁶. These demonstrated an increase in left ventricular free wall mass (due to cardiomyocyte hypertrophy), diastolic dysfunction (delayed cardiomyocyte relaxation time), increased susceptibility of cardiomyocytes to ischemia-reperfusion, increased intima-media thickness and decreased luminal area of ventricular arterioles, and decreased protein levels of HK2 (an important regulator of glucose metabolism and antiapoptotic pathways) 46 . The former three alterations were suggested, at least in part, to have been influenced by increased calcium transients in ventricular cardiomyocytes. The vascular adaptations were also found to apply to the systemic arterial system, as two month old neonatally hypoxic rats also had an elevated systolic arterial pressure (associated with a decrease in the compliance of the major conductance arteries)⁵.

These studies suggest that hypoxia in early life, as experienced by individuals with cyanotic congenital heart defects, leads to pervasive and longlasting effects on numerous physiological systems. The studies by Rohlicek et. al.^{5, 7, 8, 46} further imply that these individuals are at a significant disadvantage when performing above baseline levels. For example, during exercise an increase in myocardial oxygen demand is normally met by increasing coronary artery flow⁴⁷. This cardiac response is limited by an increase in left ventricular mass, which can reduce the diameter of the already narrowed coronary arteries, and along with systemic hypertension further increases the metabolic requirements of the myocardium^{46, 48-50}. Exercise is also accompanied by an increase in CO, which can be hindered by prolonged myocardial relaxation times^{46, 51, 52}. In adulthood, cardiac reoperation is often necessary. Decreased left ventricle contractility and increased susceptibility to ischemia-reperfusion injury may be of great consequence during these frequent adult reoperations, which may require inotropic medication and often introduce an ischemic stress. Thus, there is a great need to understand the physiological consequences of early-life hypoxia as we are preparing to treat a new and unfamiliar patient population.

Autonomic Cardiovascular Control: The Baroreflex

Several coincident patterns shared by adult 'survivors' of cyanotic congenital heart disease, the development of hypertension and cardiovascular disease, and animal models of neonatal hypoxia suggest the presence of altered autonomic cardiovascular control. Adult 'survivors' have a higher risk of morbidity and mortality, particularly as a result of cardiovascular related events⁴¹. This has been proposed to be partially a result of increased sympathetic drive,

which is strongly associated with the development of hypertension, cardiovascular disease, and sudden cardiovascular events¹⁰. Further supporting this notion is experimental evidence showing that early life exposure to hypoxia increases systemic arterial pressure at maturity⁵. The accompanying decrease in arterial compliance is also the site of afferent input to the baroreceptors; decreased afferent input as a result of reduced distensibility of the aortic and carotid baroreceptors releases sympathetic inhibition and hinders the ability of the baroreflex to regulate autonomic activity and blood pressure. In effect, more pressure is required to elicit the same degree of afferent input (*i.e.* a decrease in sensitivity). This sequence of events has already been widely studied in various models of aging. Both age and systolic blood pressure have been found to be inversely correlated to arterial compliance, baroreflex sensitivity, and sympathetic activity⁵³⁻⁵⁵. Thus, regardless of the model studied there appears to be a strong correlation between the presence of hypertension, decreased baroreflex sensitivity, and altered sympathovagal balance.

Baroreflex sensitivity can be measured using several techniques. The first quantitative measure was developed in 1969 by Smith et. al. using what is now commonly referred to as the 'Oxford' technique⁵⁶. In their original experiments, both heart rate and blood pressure were monitored while a short bolus of angiotensin was injected intravenously. The proceeding increase in blood pressure was plotted against the baroreflex-mediated decrease in heart rate (i.e. increase in R-R interval), and the slope was calculated as an approximation of baroreflex sensitivity. Although angiotensin has been replaced by other vasoactive drugs such as phenylephrine (a selective α -agonist that does not have direct effects on sinus node rhythm or cardiac contractility) and nitric oxide (a vasodilator), there remain several limitations to this widely used method. These include direct and indirect effects through sinus node activation (*i.e.* nitric oxide⁵⁷), mechanical distortion of the vasculature (*i.e.* phenylphrine⁵⁸), and activation of reflexes that oppose the baroreflex (*i.e.* cardiopulmonary stretch receptors⁵⁹). Additionally, measurement of baroreflex sensitivity from a single regression analysis may not accurately reflect normative values because of high intra-individual variability⁶⁰.

Nearly two decades after the introduction of the Oxford technique Bertineri et. al. proposed a measurement of baroreflex sensitivity calculated from spontaneous fluctuations in blood pressure⁶¹. Within the spontaneous fluctuations were sequences of directionally similar changes in blood pressure and pulse interval (PI) that were said to be baroreflex-mediated. Those sequences occurring over a minimum of three beats were plotted (systolic blood pressure [SBP] vs. PI), and baroreflex sensitivity was obtained from the average of the individually calculated slopes. Removing the baroreflex afferents (sinoaortic denervation) resulted in a drastic reduction of these sequences, confirming their baroreflex origin⁶¹. Although the Oxford and the sequence methods are not empirically equivalent, they are strongly correlated in a number of experimental and clinical settings^{62, 63}. Furthermore, the sequence technique is non-invasive, calculates baroreflex sensitivity around the physiological operating point, averages many sequences to minimize intra-individual variability, and has been shown to be reproducible in subjects over time^{64, 65}. Coupled with radio telemetry, this technique can further assess baroreflex sensitivity in awake and unrestrained animals at any time of day.

The baroreflex has traditionally been regarded as a regulator of short-term systemic arterial pressure because it has a tendency to reset (*i.e.* a shift of the sigmoidal relationship between blood pressure and heart rate) and removal of baroreceptor afferents (sinoaortic denervation) does not result in long-term elevations in blood pressure (only blood pressure variability)⁶⁶. Thus, a decrease in baroreflex function was widely believed to be a consequence and not a cause of hypertension. However, recent evidence surrounding the efferent sympathetic arm of the baroreflex and newer models of sinoaortic denervation suggests that it also contributes to the long-term control of mean arterial pressure^{22, 67}. In this regard, human epidemiological studies have found baroreflex sensitivity to be inversely correlated to current and five year ambulatory blood pressure in normotensive individuals^{55, 68} as well as current blood pressure in hypertensive patients⁶⁹. Furthermore, decreased baroreflex sensitivity may be indicative of an imbalance in global autonomic tone. This theory has been applied clinically as a prognostic

tool for stratifying patients into risk groups and predicting adverse outcomes. A low baroreflex sensitivity independently predicts poor hemodynamic status (i.e. left ventricular ejection fraction, cardiac index, wedge pressure, mitral regurgitation) and adverse outcomes (*i.e.* cardiac death, nonfatal cardiac arrest, need for transplant) in patients with heart failure⁷⁰⁻⁷², cardiac death and nonfatal acute coronary events in patients after myocardial infarction^{73, 74}, sudden death in hypertensive patients with chronic renal failure⁷⁵, all cause mortality after acute ischemic stroke⁷⁶, cardiac and cerebrovascular events in type-2 diabetic patients⁷⁷, coronary narrowing in patients with stable coronary artery disease 78 , cardiovascular risk (i.e. sympathetic activity and blood pressure) in patients with metabolic syndrome⁷⁹, and mortality, total events, and unscheduled cardiac events in postoperative congenital heart disease patients⁸⁰. In several of these studies, baroreflex sensitivity was more than a novel marker of adverse outcome. By identifying patients with additional risk factors (e.g. low heart rate variability and/or left ventricular ejection fraction), the risk for adverse outcomes was greatly increased. The baroreflex and its measure of sensitivity are evidently critical mechanisms of autonomic cardiovascular control that can help identify patients who are most at-risk.

The Present Study

Adult 'survivors' of cyanotic congenital heart disease continue to experience higher rates of morbidity and mortality long after surgical repair in infancy⁶. Neonatal hypoxia has been implicated in this as one factor involved in a range of physiological adaptations continuing throughout adulthood^{5, 7, 8, 43-46}. It is still unknown whether the increase in systemic arterial pressure in neonatally hypoxic male rats persists into maturity, and whether autonomic cardiovascular control is also affected. Furthermore, while there are sex differences in the development of hypertension and adaptations to prenatal stress^{11, 13}, our animal model has not yet considered how females respond to neonatal hypoxia. We therefore measured systemic arterial pressure at two, three, and six months in male and female neonatally hypoxic and control rats to test four hypotheses: 1)

Systemic arterial pressure remains elevated in neonatally hypoxic males throughout maturity; 2) Females respond to neonatal hypoxia differently than their male counterparts; 3) Sex steroid hormones are implicated the developmental programming of elevated blood pressure after neonatal hypoxia; and 4) Baroreflex sensitivity is altered in conjunction with elevated blood pressure after early life hypoxia.

METHODS

Animals

33 pregnant dams were purchased from Charles River (Montreal) and were received approximately 17-18 days into gestation. After birth, each litter was culled to 12 pups of mixed sex. 16 litters (hypoxic) along with their mothers were subjected to chronic hypoxia ($F_iO_2 = 0.12$) from days 1-10 of life and subsequently reared in normoxia. The remaining 17 litters (control) were reared entirely in normoxia. All animals were maintained on a 12 hour light/dark cycle (7am-7pm), with *ad lib* access to food and water.

Gonadectomy

A subset of the experimental subjects underwent gonadectomy prior to surgery. At 30 days, 8 neonatally hypoxic and 9 control males were castrated, while at 60 days 6 neonatally hypoxic and 6 control females were ovariectomized. The rats were operated on under aspetic conditions, anesthetized with isoflourane (2-4%), and given carprofen (5 mg/kg) prior to and one day following surgery. For males, an incision was made in the scrotum and the testes were exposed. Blood supply was arrested by ligating the testicular vasculature with sterile silk. The testes were subsequently removed and the incision was closed with wound clips. In females, a dorsal incision was made in the midline of the skin and two mediolateral incisions were made into the retroperitoneal wall. After the ovaries were exposed, blood supply was arrested using sterile silk and the ovaries removed. The retroperitoneal incisions were closed with sutures and the skin with wound clips.

Telemetric Recording of Systemic Arterial Pressure

139 rats were instrumented with intravascular telemetric recording probes (TA11PAC-40, Data Sciences International, St. Paul, MN) at approximately two

months of age (male hypoxic n = 13, male control n = 13; male castrated hypoxic = 8; male castrated control = 9; female hypoxic n = 7, female control n = 7), three months of age (male hypoxic n = 10, male control n = 12; female hypoxic n = 8, female control n = 8; female ovariectomized hypoxic = 6; female ovariectomized control = 6), and six months of age (male hypoxic n = 6, male control n = 7; female hypoxic n = 7, female control n = 12). The rats were instrumented under aseptic conditions, anesthetised with isoflourane (2-4%), and given carprofen (5 mg/kg) prior to and two consecutive days following surgery. A midline incision through the ventral abdominal wall was made and the descending aorta was exposed. The 0.7 mm diameter catheter was introduced into this vessel caudal to the renal arteries and rostral to the iliac bifurcation. The attached transmitter (4.4 cc) was placed in the abdominal cavity and secured to the dorsal wall of the abdomen as the cavity was sutured closed. The skin was closed with wound clips. Six days after surgery, the individually caged rats were placed on their respective receivers (RPC-1, Data Sciences International, St. Paul, MN) in a recording room with limited human traffic. On day seven following instrumentation, systemic arterial pressure and locomotor activity were continuously recorded for 48 hours at a rate of 500 Hz using Dataquest ART 4.1 software (Data Sciences International, St. Paul, MN). The catheter system used has been found to have a frequency response (-3 dB = 40 Hz) that faithfully reproduces the pressure pulse of rats with heart rates averaging 420 beats/minute (7 Hz) when compared with the recording from intravascular high fidelity catheters⁸¹. Data were further separated into active (night-time) and resting (daytime) periods.

Locomotor activity

Locomotor activity, measured in counts/minute, is a relative measure of movement calculated from changes in transmitter signal strength perceived by the receiver. Signal strength is transformed into activity counts using an algorithm dependent on the amplitude and speed of change in signal strength. Activity data were averaged for the resting (daytime) and active (night-time) periods.

Calculation of Baroreflex Sensitivity

Baroreflex sensitivity was calculated during the rats' active and resting periods using the sequence technique (adopted from Oosting⁶² and Waki⁸²). Systolic blood pressure and pulse interval were determined for all beats over thirty minute periods at noon (resting) and midnight (active) using Dataquest ART 4.1 software (Data Sciences International, St. Paul, MN). A 10-beat moving average was manually calculated for each variable and then analysed with Hemolab software (Iowa City, IA). A 3-beat delay was introduced between SBP and PI. Sequences of three or more consecutive heart beats with directionally similar changes in SBP and PI were plotted as SBP vs. PI. The slope was determined for each individual sequence with a correlation coefficient greater than 0.8, and the average of all the individual slopes was calculated. The same procedure was repeated at a delay of 4 and 5 beats, with the average of the three delays taken as an index of baroreflex sensitivity (for an illustrated example see: Appendix, Example of Baroreflex Sensitivity Calculation Using the Sequence Technique).

Statistics

All data were expressed as mean \pm S.E.M. Between-group analyses were performed using the Student's t-test. A p-value ≤ 0.05 was considered to be statistically significant. Effect size was calculated for arterial pressure in males during the active (night-time) period, where

$$Effect Size = \frac{(Mean of hypoxic group) - (Mean of control group)}{Standard Deviation}$$

All experiments were approved by the McGill University Health Center Research Institute Ethics Committee, Montreal, Canada. The Canadian Council on Animal Care (CCAC) guidelines were followed for all experiments.
RESULTS

Age and Body Weight

There was no difference in the age of the rats at the time of recording (Table 1). Two month old neonatally hypoxic males weighed significantly less than controls at recording (control: $365 \pm 9g$, hypoxic: $418 \pm 9g$, $p \le 0.05$), as did gonadectomized, neonatally hypoxic males and females (male control: $392 \pm 17g$, male neonatally hypoxic: $314 \pm 17g$, $p \le 0.01$; female control: $411 \pm 6g$, female neonatally hypoxic: $351 \pm 11g$, $p \le 0.01$). There was no difference in body weight between the groups in three or six month old males or in non-ovariectomized females at any age (Figure 1).

		Male		Female	
		Control	Hypoxic	Control	Hypoxic
2 Month	Age (days)	70 ± 0.3	70 ± 0.3	70 ± 0.5	71 ± 0.4
Gonadectomized	Age (days)	63 ± 0.9	63 ± 0.9	99 ± 1	99 ± 1
3 Month	Age (days)	104 ± 0.8	104 ± 0.9	105 ± 0.6	105 ± 0.6
6 Month	Age (days)	190 ± 2	190 ± 2	191 ± 3	192 ± 2

Table 1. Age of male and female neonatally hypoxic and control rats

Data are presented as mean value \pm S.E.M.



Figure 1. Weight of male (blue) and female (orange) control and neonatally hypoxic groups before recording at two, three, and six months of age. CAS = castrated, OVX = ovariectomized; Data are presented as mean value \pm S.E.M.; p \leq 0.05.

Systemic Arterial Pressure

In the male neonatally hypoxic rats SBP and MAP were significantly higher during the active (night-time) period at all ages studied (2, 3, and 6 months of age) compared to controls. Additionally, at three months of age DBP was significantly greater than in control animals during the active (night-time) period. During the resting (daytime) period SBP and MAP were significantly higher in neonatally hypoxic rats aged three and six months of age compared to controls. Following castration at 30 days of age two month old male rats also had a significantly higher SBP compared to controls. No significant differences in systemic arterial pressure were observed between neonatally hypoxic and control intact females at any age or after ovariectomy (Figure 2; Appendix, Tables 2-9).



Figure 2. Active (night-time) systemic arterial pressure of male (blue) and female (orange) control and neonatally hypoxic rats at two, three, and six months of age. CAS = castrated, OVX = ovariectomized; Data are presented as mean value \pm S.E.M.; a = systolic pressure, b = mean pressure, c = diastolic pressure; p \leq 0.05.

Heart Rate

There were no differences in heart rate between neonatally hypoxic and control male rats at any age or in two month old female rats. Heart rate was mildly but significantly decreased in three and six month old neonatally hypoxic females compared to controls during the active and resting period, respectively (two month neonatally hypoxic: 415 ± 6 beats/min, two month control: 441 ± 9 beats/min; three month neonatally hypoxic: 331 ± 6 beats/min, three month control: 349 ± 5 beats/min; Appendix, Tables 2-9).

Locomotor Activity

Locomotor activity was not different between neonatally hypoxic and control rats at any age or time of day, except for increased activity in three month old neonatally hypoxic male rats compared to controls during the active (nighttime) period (Figure 3; Appendix, Tables 2-9).



Figure 3. Locomotor activity of male (left) and female (right) control and neonatally hypoxic rats at two, three, and six months of age. Resting (light bar) and active (dark bar) periods are depicted. CAS = castrated, OVX = ovariectomized; Data are presented as mean value \pm S.E.M.; a = active (night-time) period; p \leq 0.05.

Effect size

As noted above, systolic pressure was increased in neonatally hypoxic males at two, three, and six months of age. Calculation of the effect size in these animals indicated an important consequence of neonatal hypoxia at all ages. Figure 4 shows the difference in systolic blood pressure between neonatally hypoxic and control rats (along the y-axis) with effect size listed within the graph. The effect size of hypoxia in early life on night-time SBP was 0.82 (95% confidence interval [CI] = 0-1.59), 1.16 (95% CI = 0.21-2.02), and 1.91 (95% CI = 0.49-3.06) at two, three, and six months, respectively (Appendix, Table 10). Due to the large confidence intervals it was not possible to reliably determine if effect size increased with age given the sample numbers.



Figure 4. Active (night-time) difference in systolic arterial pressure between neonatally hypoxic and control male (blue) and female (orange) rats at two, three, and six months of age. Effect sizes with 95% confidence intervals are listed within the male delta bars.

Baroreflex sensitivity

Baroreflex sensitivity was significantly decreased in the two month neonatally hypoxic males compared to controls during the active (night-time) period (hypoxic: 0.93 ± 0.09 ms/mmHg, control: 1.30 ± 0.16 ms/mmHg, p-val = 0.054; Figure 5; Appendix, Table 11). A similar trend that did not achieve significance was observed during the resting period (hypoxic: 1.27 ± 0.10 ; control: 1.43 ± 0.11). Baroreflex sensitivity was not significantly different in three or six month neonatally hypoxic males compared to controls (Figure 5; Appendix, Table 11).



Figure 5. Baroreflex sensitivity during the active (night-time) period in control (red) and neonatally hypoxic (blue) male rats at two, three, and six months of age. Data are presented as mean \pm S.E.M.; p \leq 0.05

DISCUSSION

Summary of Results

The work presented here confirms previously published results indicating elevated systemic arterial pressure in two month male rats hypoxic in early life compared to control⁵. We have further found that this effect does not disappear with age but persists later into maturity with substantial effect sizes evident. The adult hypertensive effect of neonatal hypoxia was not present in females.

In examining the role of sex hormones, we found that castration at 30 days of age had no effect on male blood pressure at two months of age. In females, ovariectomy failed to unmask the programming effect of neonatal hypoxia on blood pressure at three months of age.

Baroreflex sensitivity, a measure of cardiovagal control, was significantly decreased in the two month neonatally hypoxic males compared to controls during their active (night-time) period. A similar but non-significant decrease in sensitivity was also observed in three month old neonatally hypoxic males. By six months of age, neonatally hypoxic and control males had similar baroreflex sensitivities.

Persistence of Elevated Blood Pressure into Maturity

The developmental programming of elevated blood pressure is a common feature amongst many models of pre- and postnatal stress (reviewed by Thronburg³, Nuyt⁸³, Ojeda⁸⁴, Grigore¹³, McMillen², and Vehaskari⁴). Our data suggest the effects of neonatal hypoxia on the arterial vasculature, and by association systemic arterial pressure, are present at two months of age in the male rat and do not progress thereafter. The most notable and consistent increase in our neonatally hypoxic males was systolic blood pressure. While there are several variables that determine this, a chronic increase is likely the result of decreased compliance of the major conductance arteries. In this regard, the

laboratory of Rohlicek has previously reported increased aortic pulse wave velocity (a strong indicator of decreased compliance) in neonatally hypoxic male rats compared to controls at two months of age⁵. This is consistent with reports of structural and functional vascular dysfunction common amongst several models of developmentally programmed hypertension⁸³. Longitudinal studies following early life stress have likewise shown a consistent difference in systemic arterial pressure between experimental animals and controls up to six months of age^{85,88}. Thereafter, exacerbation of the hypertensive effect has been reported in some^{85,88}, but not all⁸⁶, studies. This worsening may primarily be a consequence of prolonged exposure to increased luminal pressure in the vasculature rather than the continued, direct influence of prenatal stress⁸⁹.

Sex Differences in Blood Pressure Regulation

A myriad of clinical and experimental studies on the evolution of blood pressure with age and the developmental programming of hypertension strongly indicate a role for sex hormones. In a cohort of 300 healthy boys and girls studied between the ages of 10 and 18, systolic blood pressure increased throughout puberty in males but not in females²⁸. Throughout middle age, men have been found to have a higher systolic blood pressure than females^{90, 91}. Another study of 352 healthy males and females aged 20-79 without a history of cardiovascular or renal diseases also found that until the age of 60, males continued to have elevated systolic blood pressure compared to age-matched, premenopausal women²⁷. This pattern declined with the onset of menopause and was no longer apparent in the 70-79 age bracket. Thus, significant periods of hormonal changes, such as puberty and menopause, are coincident with differences in blood pressure profiles between males and females. Two hypotheses, albeit simple, can be put forth from these observations: 1) testosterone contributes to elevated blood pressure, and 2) estrogen protects against elevations in blood pressure. Extensive data in this field are reviewed below from two different perspectives. The first examines the role of sex steroid hormones (SSHs) in rodent and human models of essential hypertension (e.g. postmenopausal women, spontaneously hypertensive rats, or Dahl salt-sensitive rats). The second links this to animal models of pre- or postnatal stress that lead to the developmental programming of hypertension (*e.g.* rat offspring of intrauterine growth restricted dams). Although there are inconsistencies in the literature, a recurring pattern of renal and renin-angiotensin dysfunction influenced by SSHs emerges.

Sex Steroid Hormones in Human and Animal Models of Essential Hypertension

Current evidence suggests a major agonistic role for testosterone and either no role or a minor antagonistic one for estrogen regarding the development of hypertension. Reckelhoff et. al. measured systemic arterial pressure using tailcuff plethysmography in male and female spontaneously hypertensive rats (SHRs) who had undergone sham surgery (intact), castration, ovariectomy, or ovariectomy plus testosterone⁹². Systolic blood pressure was highest in the intact male SHRs and significantly lower in intact female SHRs. Castration reduced systolic blood pressure to levels observed in intact females. Ovariectomy did not result in an increase in blood pressure, and addition of testosterone to ovariectomized females significantly increased blood pressure. In this study testosterone directly increased systolic blood pressure while a lack of estrogen did not appear to result in any increase. While the majority of evidence suggests testosterone contributes to elevations in blood pressure, it is difficult to find consistent results regarding the effects of estrogen. In several studies of Dahlsensitive and Sprague Dawley rats, ovariectomy significantly increased blood pressure when measured via tail cuff, chronic indwelling catheter, or radio telemetry⁹³⁻⁹⁵. When estrogen was added back to the ovariectomized females, blood pressure dropped to pre-surgical intervention levels^{94, 95}. This is in contrast to tail-cuff and radio telemetry studies on Sprague Dawley and SHRs performed by Reckelhoff and Ojeda^{92, 96, 97}. In humans, this is most readily compared to hormonal replacement therapy in postmenopausal women. Dubey et. al. reviewed several reports and found that estrogen replacement had no effect, a minimal effect, a significant effect, or a significant effect only during the night-time on blood pressure⁹⁸. The studies in question are further complicated by varying dosages, mode of delivery, duration of therapy, type of estrogen treatment, and method of blood pressure recording. Timing of therapy has also been suggested to be a critical determinant of efficacy as hormone replacement may not be able to reverse permanent atherosclerotic damage⁹⁹. Similarly, comparisons between animal studies remain difficult as various strains of rats display differing responses to ovariectomy, and various methods of recording blood pressure may contribute to inter-study variability.

Targets of Sex Steroid Hormones: The Renal and Renin-Angiotensin Systems

The renal system, in particular pressure-natriuresis and the RAS, appears to be a common target of SSHs when elevated blood pressure is present¹¹. A rightward shift in the arterial pressure vs. sodium excretion (pressure-natriuresis) relationship is involved in most forms of essential hypertension^{100, 101}. In SHRs, intact males or ovariectomized females given testosterone exhibit a greater rightward shift in the pressure-natriuresis relationship than castrated males, ovariectomized females, or intact females⁹². Furthermore, transplantation of male SHR kidneys into female SHRs, or the reverse, does not alter blood pressure¹⁰². This generally goes against the convention that "hypertension follows the kidneys"^{100, 103}. These experiments suggest an additional extrinsic factor, likely testosterone, is associated with impaired pressure-natriuresis and elevated blood pressure in male SHRs. Although the link between testosterone and impaired renal function has not been elucidated, there is a positive relationship between the former and renal angiotensinogen mRNA, plasma renin activity, and blood pressure^{11, 104, 105}. This suggests testosterone stimulates the RAS, ultimately leading to elevated Ang-II, altered pressure-natriuresis, and increased blood pressure. Reckelhoff et. al. explored this possibility in SHRs given enalapril, an angiotensin converting enzyme inhibitor⁹⁶. After treatment, blood pressure decreased in the five groups of SHRs (intact male, castrated male, intact female, ovariectomized female, and ovariectomized female with testosterone) to equivalent levels. In all groups the elevation in blood pressure, regardless of sex, was mediated by the RAS; however, the larger decrease in SHR males compared to females strongly suggests testosterone promotes hypertension via the RAS^{11, 96}.

Sex Steroid Hormones in Animal Models of Developmentally Programmed Hypertension

In the same way that SSHs have been implicated in the various models of essential hypertension, testosterone has been suggested to promote the developmental programming of hypertension after pre- or postnatal stress while estrogen appears to provide mild to moderate protection. Ojeda and colleagues have shown that male rat offspring of intrauterine growth restricted (IUGR) dams have greater testosterone levels and blood pressure than controls at maturity, a relationship that is abolished after castration⁸⁷. Again, this increase appears to be mediated by the RAS and exacerbated by testosterone, since enalapril decreases mean arterial pressure in both intact and castrated IUGR rats to similar levels (i.e. to a greater extent in intact IUGR than castrated)⁸⁷. In a parallel study, Ojeda *et*. al. found that female IUGR offspring had similar blood pressures to controls; however, after ovariectomy blood pressure increased only in the rats who had experienced IUGR⁹⁷. Estrogen replacement after ovariectomy decreased blood pressure in the ovariectomized IUGR females, as did enalapril. This suggests estrogen protects against elevations in blood pressure after IUGR. Moreover, the mechanism of estrogen protection was mediated by the vasodilatory branch of the RAS⁹⁷. After IUGR, levels of ACE (upstream of Ang-II, a vasoconstrictor) and ACE2 (upstream of Ang-1-7, a vasodilator) were both increased in females, although the balance was skewed towards elevated ACE2 expression. After ovariectomy, only ACE remained significantly elevated, suggesting that ovaries were required for the increased expression of the vasodilatory ACE2 pathway after IUGR⁹⁷. In these models of prenatal stress, males developed a testosterone dependent increase in blood pressure, while females with intact ovaries were protected. Not all models of perinatal stress demonstrate female protection. On the contrary, they appear only to be protected against mild to moderate stresses in early life. Woods *et. al.* demonstrated that although a 5% low protein diet given to pregnant dams resulted in elevated blood pressure in both male and female offspring, a modestly low protein diet of 9% resulted in elevated blood pressure only in males¹⁰⁶⁻¹⁰⁸. Thus, if severe enough, both sexes suffer from similar long-term consequences.

Removal of Sex Steroid Hormones and the Neonatal Hormonal Milieu

There appear to be similar targets when hypertension (essential or developmentally programmed) is present: elevated testosterone, impaired renal function, and increased RAS activity in males; moderate protection in females. Our results indicating a sex-dependent increase in blood pressure after neonatal hypoxia suggest a similar pattern of physiological adaptations that are influenced by sex; however, castration did not prevent an elevation in blood pressure after neonatal hypoxia. Not all models of perinatal stress are equivalent, as others have shown that different models of perinatal stress can produce hypertension that is similarly independent of castration at 30 days¹⁰⁹. This suggests: 1) testosterone effects the development of hypertension after some but not all perinatal stresses, 2) testosterone does not affect the developmental programming of hypertension after neonatal hypoxia, or 3) the programming effects of testosterone are completed before 30 days. Additionally, ovariectomy did not reveal the hypertensive effect of neonatal hypoxia. As reviewed above, ovariectomy has produced varying results in hypertensive models which depend on the animal model and stress (*i.e.* type, timing, and severity). Our limited sample size of ovariectomized females makes interpretation beyond this difficult. It is possible that our model of hypoxia was too mild to impart phenotypic changes in systemic arterial pressure, and/or gonadectomy was not performed early enough in either sex. In this regard, neonatal gonadectomy may be more appropriate. Several studies have discovered sex differences which are modulated by SSHs during neonatal development. For example, newborn rats display sex differences in the hypothalamic-adrenal-pituitary axis that range from adrenocortical secretions to gene expression^{110, 111}. After neonatal gonadectomy, some of these differences were abolished while several that only develop in adulthood were prevented^{110, 111}. Another study found that the number of premature ventricular complexes (PVCs) after perinatal hypoxia ($F_iO_2 = 0.12$) decreased in male rats compared to controls, and was further decreased after neonatal castration¹¹². The number of PVCs in females was not affected by perinatal hypoxia or neonatal ovariectomy¹¹². An earlier study by the same authors using intermittent hypobaric hypoxia during the perinatal period without neonatal gonadectomy produced opposite results¹¹³, highlighting the impact that different methodologies can have. In regards to blood pressure, the development of hypertension in Dahl salt-sensitive and SHRs is delayed or prevented after neonatal castration¹¹⁴⁻¹¹⁶. Existing literature on the significance (or lack thereof) of neonatal SSHs on the development of hypertension after perinatal stress is very limited, despite numerous reports of sex differences related to these phenomena. Neonatal gonadectomy in perinatally hypoxic ($F_iO_2 = 0.12$) rats prevented an elevation in systemic arterial pressure in both males and females, whereas it significantly increased ventricular weight and muscularization of the pulmonary vasculature only in perinatally hypoxic females compared to controls¹¹⁷. Another paper examined the effects of neonatal gonadectomy on the development of hypertension in male but not female rats after postnatal overfeeding¹¹⁸. In this case, neonatal castration prevented an increase in blood pressure, whereas neonatal ovariectomy had no effect (i.e. blood pressure of overfed and control females were no different from each other before or after ovariectomy). Once more, the lack of effect seen after ovariectomy may be a consequence of the relatively mild stress imposed. To the best of our knowledge, there are no studies to date examining the direct role of SSHs during the neonatal period on the development of the RAS in adulthood. Nevertheless, renal development during the neonatal period is sensitive to disturbances of the RAS, resulting in decreased renal function that is more apparent in males than females. Directly antagonizing AT₁ receptors (which bind Ang-II) during neonatal development increases blood pressure and decreases nephron number equally in both males and females, while it either only or more severely affects

males via decreases in GFR and increases in glomerular hypertrophy, glomerulosclerosis, interstitial fibrosis, and albuminuria^{119, 120}. With increasing age, these disparities are amplified¹¹⁹. Coincidently, animal models of perinatal stress have shown similar renal dysfunction that is also more substantial in males², ¹²¹. Although speculative, it is possible that neonatal SSHs are also implicated in sex-dependent alterations of the renal system that ultimately lead to the programmed hypertension seen in many models of perinatal stress, including neonatal hypoxia.

Autonomic Cardiovascular Control

Concurrent with increased systemic arterial pressure, we found baroreflex sensitivity to be significantly decreased in neonatally hypoxic males at two month of age compared to controls. This may be partially explained by reduced compliance of the major conductance arteries after neonatal hypoxia³. Impaired distensibility of stretch-activated baroreceptors within the aortic arch and carotid sinus necessitate greater intramural pressure to achieve similar levels of afferent activity (in the absence of baroreflex resetting). An inverse correlation between baroreflex sensitivity and compliance has also been observed in trained athletes and the elderly, whom have increased and decreased compliance, respectively⁵³, ⁵⁴. Involvement of the baroreflex in our model of elevated blood pressure after neonatal hypoxia is a separate and complex question. The continued presence of elevated blood pressure in neonatally hypoxic males argues against a return of arterial compliance to control levels at three or six months of age, even though baroreflex sensitivity tended to return to control levels at this time. An important consideration in our analysis is the physiological meaning of values calculated by sequence method. Notwithstanding its clinical significance, measurements of baroreflex sensitivity (when calculated from changes in blood pressure and heart rate) predominantly quantify the cardiovagal component of the baroreflex despite sympathetic and parasympathetic innvervation of the sinus node. This can be attributed to the relative speed and dominance with which the parasympathetic system modifies heart rate compared to the sympathetic system. Muscarinic (M_2) receptors in the SA node signal through GPCRs that are in direct contact with hyperpolarizing K_{ACh} channels. In humans, this means a baroreflex vagal response time of less than 500 msec¹²². Conversely, sympathetic cardiac signalling through β_1 -adrenergic receptors relies on the production of the second messenger cAMP and its degradation by phosphodiesterases. Accordingly, its conduction has been estimated to be around 2-3 seconds¹²³. Given that the sequence method of calculating baroreflex sensitivity uses a relatively short delay between pulse interval and systolic blood pressure, it is not surprising that atropine (a cardiac parasympathetic antagonist) decreases sensitivity and the number of baroreflex sequences to a much greater degree than metoprolol or propranolol (cardiac sympathetic antagonists)^{62, 65}.

Nevertheless, both branches of the baroreflex must be considered. As reviewed in the Introduction, decreased baroreflex sensitivity is strongly associated with elevated sympathetic activity and increased cardiovascular risk. Without direct recordings of sympathetic nerve activity, we can only infer that decreased sensitivity in neonatally hypoxic males was accompanied by increased sympathetic outflow at two months of age. Whereas cardiovagal control seems to return by six months of age, sympathetic activity may still remain elevated.

Until recently, any notion of long-term regulation of pressure by the baroreflex was discounted in support of Guyton's renal-centric model¹⁰¹. In 1956, McCubbin *et. al.* were the first to demonstrate a return of baroreceptor afferent responsiveness in the face of chronic hypertension¹²⁴. Thereafter, Cowley *et. al.* determined that the baroreflex was only relevant for short-term fluctuations in blood pressure. 24-hour ambulatory recordings from sinoaortic denervated dogs revealed that an initial increase in mean arterial pressure disappeared within several days, whereas the variability of pressure around the mean continued to be greatly elevated⁶⁶. Sinoaortic denervation remained the preferred method of studying diminished baroreflex activity for the next three decades despite its inability to simulate reduced (as opposed to absent) afferent activity. This methodological limitation was overcome in 2002 with a new model of chronic

baroreceptor unloading⁶⁷. This was achieved by denervating baroreceptors within the aortic arch and one carotid sinus while ligating the common carotid artery proximal to the remaining carotid sinus. What followed was a drop in carotid sinus pressure (relative to systemic arterial pressure) and activation of the remaining baroreceptors. Mean arterial pressure increased by approximately 20 mmHg for seven days, which was sufficient to elevate carotid sinus pressure to pre-occlusion levels (a connection between the circle of Willis and the distal portion of the common carotid artery in dogs enabled systemic arterial pressure to influence carotid sinus pressure)⁶⁷. This contradicts earlier reports of rapid and complete baroreceptor resetting after sinoaortic denervation in several species¹²⁵ ¹²⁹. With decreased activity to the NTS, the CVLM loses inhibitory control of the RVLM and its presympathetic motor neurons; however, after several days without any afferent input to the NTS, significant rewiring of the central baroreflex pathway occurs. A new, unidentified source of GABA inhibits tonic NTS activity, while the CVLM regains inhibitory control of the RVLM in an NTS-independent manner^{130, 131}. This may explain why mean arterial pressure but not blood pressure variability returns to normal within several days of sinoaortic denervation²².

Chronic baroreceptor unloading (artificially decreasing afferent activity) has demonstrated the baroreflex's ability to maintain elevated blood pressure (up to five weeks in one study¹³²). In contrast, a return of normal afferent baroreceptor activity (*i.e.* an increase in baroreceptor threshold) or heart rate responses to pressure changes in chronic hypertension has repeatedly been equated to complete baroreflex resetting^{124, 133, 134}; unfortunately, these criteria for resetting do not wholly encompass autonomic baroreflex function. For instance, Ang-II induced hypertension leads to adaptation of the baroreflex response curve relating mean arterial pressure to heart rate (*i.e.* a rightward shift) and sustained inhibition of renal sympathetic nerve activity (*i.e.* preservation abolished suppression of renal sympathetic nerve activity¹³⁶. If heart rate or afferent baroreceptor traffic were the only consideration in these studies, some would have argued that complete

baroreflex resetting had occurred. Although speculative, it is conceivable that in neonatally hypoxic male rats, the sympathetic branch of the baroreflex remains elevated long after the cardiovagal branch becomes similar to controls.

METHODOLOGY

Hypoxia

Constant normobaric hypoxia (FiO₂ = 0.12) was achieved by mixing a continuous source of air and nitrogen so as to avoid repetitive reoxygenation. The degree of hypoxia has been optimized to produce significant physiological changes while maximizing rat pup survival^{5, 7, 8, 46}. Although we were not able to directly measure arterial oxygen saturation, indirect estimates using the alveolar gas equation suggest arterial oxygen saturation to be around 60% (see Appendix, Alveolar Gas Equation). A duration of 10 days (from days 1-10 of life) was chosen as it represents a significant portion of early development (rat pups are weaned at day 21 of life and begin sexual maturity at approximately 32-34 days in females and 45-48 days in males¹³⁷.

Telemetry

Many factors were considered in our decision to use radio telemetry over alternative methods of blood pressure recording. Indirect measurement using tailcuff plethysmography does not require surgery and is relatively inexpensive, making it desirable for high throughput studies. However, it also requires animals to be warmed (to increase blood flow through the tail vein) and restrained, eliciting a significant stress response that is not always comparable between experimental groups¹³⁸. Furthermore, intermittent measurements of blood pressure provide little context considering the significant variance of blood pressure over a 24 hour period¹³⁹. Blood pressure can also be measured directly with exteriorized fluid-filled catheters connected tether/swivel system. These reliably and accurately measure blood pressure in conscious animals but may also inhibit mobility and/or generate stress¹³⁹. Postoperative care is also required to prevent infection of the exteriorized site and clotting of the catheter. Radio telemetry, despite a large capital investment, provides the most advantages for the continuous and direct measurement of blood pressure in freely moving, conscious animals. Although invasive surgery is required, animal weight, circadian rhythms, and blood pressure return to normal within 5-7 days after¹⁴⁰.

Confounding variables, such as animal weight and activity, likely did not contribute to our results. Activity was similar in nearly all experimental groups despite significant differences in systemic blood pressure. Only three month neonatally hypoxic males had significantly higher locomotor activity than controls. Since there was no pattern associated with systemic blood pressure and activity across the other ages in males or females, it is likely that the three month males were an anomaly. It is further possible that hypoxia disrupted the quantity or composition of milk produced by the lactating dams which could have lead to decreased weight. In order to assess the contribution of decreased weight and catch up growth that is experienced by neonatally hypoxic rats, our laboratory used a model of growth restriction utilizing increased litter size. This technique produces rat pups of similar weight to those experiencing neonatal hypoxia^{5, 141}. At 2 months of age, blood pressure was not different between growth-restricted and control rats⁵. Thus, the severity and timing of growth-restriction experienced by our neonatally hypoxic rats does not contribute to increased systemic arterial pressure. Regarding composition, hypoxia has been shown to decrease the concentration of epidermal growth factor in both maternal milk supply and rat pup stomach contents¹⁴². However, only 1% of orally administered epidermal growth factor is absorbed into the plasma and peripheral organs of rat pups¹⁴³. An additional concern was the effect of the estrous cycle in females on systemic arterial pressure. Telemetric measurements have shown that systemic arterial pressure increases only marginally (1-2 mmHg) during proestrus compared to the rest of the cycle¹⁴⁴. This is considerably less than the increase in systemic arterial pressure seen in our male animals following neonatal hypoxia.

Baroreflex sensitivity

Several modifications to the sequence method were applied in this study due to considerably faster heart rates in rats (approximately 400 beats/min) compared to humans. Consistent with more recent variations of the sequence method for the rat, we chose not to have a pressure threshold of 1 mmHg for changes in systolic blood pressure or 4 ms for changes in pulse interval as was utilized in the original human studies^{62, 82}. Additionally, a 10-beat moving average (*i.e.* low-pass filter) was applied to raw arterial pressure and pulse interval data in order to minimize respiratory-related mechanical fluctuations⁶². Finally, several baroreflex sensitivity values were averaged after applying delays of 3, 4, and 5 beats between the first systolic blood pressure point and the subsequent pulse interval because of the transmission time required by the baroreflex⁶². These variations have been empirically correlated to best fit with established methods (i.e. the Oxford technique, as well as several other frequency domain methods also calculated from spontaneous fluctuations^{1, 62, 82, 145}). Also consistent with the vast majority of previous studies employing the sequence method, systolic blood pressure was used to calculate baroreflex sensitivity as this correlates best to other established methods of measuring baroreflex sensitivity¹⁴⁵.

FUTURE DIRECTIONS

Neonatal Gonadectomy

We have found that persistent elevations in systemic arterial pressure after neonatal hypoxia occur in a sex-dependent manner. This hypertensive effect is not explained by an adult sex-steroid effect as evident from our experiments involving adult gonadectomy. Male-female divergences in the development of the hypothalamic-pituitary-adrenal axis and renal/renin-angiotensin system are apparent at birth and can be modified by neonatal manipulations^{110, 111}. One possibility is that sex steroid hormones may play a role in blood pressure programming earlier than we accounted for in the present study. In this regard, neonatal gonadectomy may offer greater insight into the sex dependence of the adult hypertensive effect of neonatal hypoxia.

Interference of Renin-Angiotensin Signalling

Dysfunction of the renin-angiotensin system is invariably coexistent with many models of elevated blood pressure. The therapeutic potential of blocking the renin-angiotensin system is diverse, and has been shown to increase baroreflex sensitivity and heart rate variability while reducing blood pressure, morbidity, and mortality in hypertensive humans^{146, 147}. Animal studies have shown sex-dependent elevations in renin-angiotensin system activity and blood pressure after prenatal stress that can be reversed by inhibiting the formation of Ang-II^{87, 97}. The relative importance of this mechanism in our model could be gauged by interfering with renin-angiotensin system signalling.

Renal Sympathetic Nerve Activity and Heart Rate Variability

Coincident with elevated systemic arterial pressure, autonomic cardiovascular control was decreased in neonatally hypoxic males at two months of age. Over time the magnitude of this decrease relative to controls diminished. Nevertheless, experimental models of decreased baroreflex sensitivity have shown that an initial decrease in cardiovagal baroreflex sensitivity can be followed by recovery of one autonomic branch but not the other¹³⁵. Measuring renal sympathetic nerve activity would give a complementary view of the sympathetic arm of the baroreflex that cannot be examined from measurements of systemic arterial pressure alone. Similarly, heart rate variability (HRV) represents an alternative quantification of autonomic activity and is an independent predictor of numerous adverse cardiovascular events. Variations in R-R interval (not heart rate) are taken from electrocardiographic recordings. These are analyzed in the time and/or frequency domain and provide an approximation of overall autonomic activity and the relative levels of parasympathetic and sympathetic balance¹⁴⁸ (assuming cardiac responsiveness is normal¹⁴⁹). Generally, a low HRV indicates a loss of autonomic cardiovascular control¹⁵⁰. In conjunction with other measure of autonomic activity such as baroreflex sensitivity, HRV may provide further insight into autonomic cardiovascular control after neonatal hypoxia.

Prolonged Telemetric Recording of Systemic Arterial Pressure

Whether or not a decrease in baroreflex sensitivity precedes an increase in blood pressure in our experimental model is still unknown. Other models of prenatal stress have shown altered baroreflex function before the onset of hypertension¹⁵¹. Decreased baroreflex sensitivity has also been found to predict an unfavourable 5-year evolution of blood pressure in a normotensive human population⁶⁸. Telemetric recording of systemic arterial pressure over several months in the same rat may be able to identify when these alterations are occurring.

Recording of Systemic Arterial Pressure in Patients with Cyanotic Congenital Heart Disease

Ultimately, our hypotheses need to be tested in adults with cyanotic congenital heart disease. Ambulatory blood pressure monitoring could give meaningful insight into blood pressure patterns and baroreflex sensitivity in these individuals.

CLINICAL IMPLICATIONS

Controlled experimental conditions have permitted us to assess the effects of a single and constant variable, hypoxia, on the subsequent development of systemic arterial pressure and autonomic cardiovascular control at maturity in rats. Even though direct comparisons between rat and human cardiovascular physiology cannot be made, these data provide greater insight into the contributing factors that may lead to an increased late hazard risk in those with cyanotic congenital heart disease. The present study raises the possibility that these patients may have elevated systemic blood pressure and compromised autonomic cardiovascular control. A lack of ambulatory blood pressure data for these patients, and the known risks associated with hypertension and decreased baroreflex sensitivity emphasize the need for more clinical research in this area.

The treatment of cyanotic congenital heart disease is continually evolving. Advances in surgical technique have drastically improved perioperative survival, and yet a significant late hazard risk remains⁶. Our data support the growing body of evidence implicating neonatal hypoxia as one factor contributing to this phenomena^{5, 7, 8, 43-46}. It also suggests earlier repair and relief of prolonged hypoxemia through complete surgical repair in infancy may contribute to improved physiological function and/or outcome later in life. Indeed, evidence of increased immediate survival and speed of recovery after complete repair before twelve months of age complements this hypothesis¹⁵².

Only recently have investigators examined the influence of sex on the prognosis of congenital heart disease. Several authors have reported a higher incidence of adulthood reoperation, mortality after reoperation, and overall mortality in men compared to woman with congenital heart diseases¹⁵³⁻¹⁵⁵. Our data demonstrating sex differences in the development of hypertension after neonatal hypoxia support these observations of male susceptibility. Taken

together, it is likely that gender differences in congenital heart diseases are more important than previously appreciated.

CONCLUSION

The effects of neonatal hypoxia on several aspects of adult cardiovascular physiology in the rat have been tested with the following results: 1) Systemic arterial pressure remains elevated in neonatally hypoxic males throughout maturity; 2) Neonatal hypoxia does not program elevated blood pressure in adult females; 3) A lack of sex steroid hormones one month prior to telemetric recording of systemic blood pressure does not affect neonatally hypoxic or control males, nor does it unveil the developmental programming effects of neonatal hypoxia in females; and 4) Baroreflex sensitivity is decreased in conjunction with elevated blood pressure in two month male rats after early life hypoxia.

While our data suggest the mechanism of programming by neonatal hypoxia is independent of sex steroid hormones, they do not rule out direct gene effects (*i.e.* regulation of y-chromosome genes) or programming by sex steroid hormones during the neonatal period. Further studies need to be conducted to determine the mechanisms resulting in sex-dependent programming by neonatal hypoxia. Decreased baroreflex sensitivity indicates impaired autonomic cardiovascular control (specifically cardiovagal control and indirectly sympathetic activity) in the early stages after neonatal hypoxia in males. Despite recovery of the cardiovagal branch by six months of age, increased sympathetic activity may remain. The location of these changes is also unknown, as baroreflex sensitivity ultimately reflects the final output of a long reflex loop, and is not a direct measure of total autonomic activity. Changes to any part of the reflex loop are plausible, including mechanical stretch baroreceptors, afferent transmission, central processing, efferent transmission, or effector organs.

Neonatal hypoxia in the rat results in significant physiological adaptations in adulthood that are sex-specific. A greater understanding of the physiology underlying these changes will ultimately benefit those experiencing similar stresses in early life, such as the large population of individuals with cyanotic congenital heart disease.

APPENDIX

Supplemental Data

Table 2. Systemic arterial blood pressure, heart rate, and locomotor activity during active (night-time) and resting (daytime) periods in neonatally hypoxic and control male rats at two months of age

	Active (N	ight-time)	Resting (Daytime)	
	Control $(n = 13)$	Hypoxic (<i>n</i> = 13)	Control $(n = 13)$	Hypoxic (<i>n</i> = 13)
Systolic arterial pressure (mm Hg)	124 ± 3	131 ± 2 *	119 ± 3	124 ± 1
Diastolic arterial pressure (mm Hg)	82 ± 2	86 ± 1	77 ± 1	79 ± 1
Mean arterial pressure (mm Hg)	100 ± 2	106 ± 1 *	95 ± 2	99 ± 1
Pulse pressure (mmHg)	42 ± 2	45 ± 1	42 ± 1	45 ± 1
Heart rate (beats/min)	396 ± 9	415 ± 5	335 ± 5	332 ± 5
Locomotor activity (counts/min)	4.1 ± 0.4	4.1 ± 0.3	0.8 ± 0.1	0.7 ± 0.1

Table 3. Systemic arterial blood pressure, heart rate, and locomotor activityduring active (night-time) and resting (daytime) periods in castratedneonatally hypoxic and control male rats at two months of age

	Active (N	ight-time)	Resting (Daytime)		
	Control	Hypoxic	Control	Hypoxic	
	(n=9)	(n=8)	(n=9)	(n=8)	
Systolic arterial pressure (mm Hg)	124 ± 2	131 ± 2 *	119 ± 3	122 ± 2	
Diastolic arterial pressure (mm Hg)	87 ± 1	87 ± 1	81 ± 2	79 ± 1	
Mean arterial pressure (mm Hg)	104 ± 2	107 ± 2	98 ± 2	99 ± 2	
Pulse pressure (mmHg)	38 ± 1	44 ± 1 *	38 ± 1	43 ± 1 *	
Heart rate (beats/min)	429 ± 4	429 ± 7	358 ± 6	348 ± 6	
Locomotor activity (counts/min)	4.9 ± 0.5	5.5 ± 0.4	1.2 ± 0.2	1.0 ± 0.1	

Table 4	. System	nic arterial b	lood j	pressure,	heart rate,	and loce	omoto	or activity
during	active	(night-time)	and	resting	(daytime)	periods	in r	neonatally
hypoxic	and co	ntrol male rat	ts at t	hree moi	nths of age			

	Active (N	Night-time)	Resting (Daytime)	
	Control	Hypoxic	Control	Hypoxic
	(n = 12)	(n = 10)	(n = 12)	(n = 10)
Systolic arterial pressure (mm Hg)	134 ± 2	140 ± 2 *	128 ± 1	133 ± 2 *
Diastolic arterial pressure (mm Hg)	90 ± 1	95 ± 2 *	84 ± 1	88 ± 2
Mean arterial pressure (mm Hg)	109 ± 2	114 ± 2 *	103 ± 1	107 ± 2 *
Pulse pressure (mmHg)	44 ± 1	46 ± 1	44 ± 1	45 ± 1
Heart rate (beats/min)	394 ± 7	401 ± 6	320 ± 6	313 ± 6
Locomotor activity (counts/min)	3.6 ± 0.2	4.5 ± 0.4 *	0.6 ± 0.1	0.6 ± 0.1

Table 5	. System	nic arterial b	lood j	pressure,	heart rate,	and loco	omoto	r activity
during	active	(night-time)	and	resting	(daytime)	periods	in n	eonatally
hypoxic	and co	ntrol male rat	ts at s	ix month	s of age			

	Active (N	light-time)	Resting (Daytime)	
	Control $(n = 7)$	Hypoxic $(n = 6)$	Control $(n = 7)$	Hypoxic $(n = 6)$
Systolic arterial pressure (mm Hg)	137 ± 2	146 ± 2 *	131 ± 2	141 ± 2 *
Diastolic arterial pressure (mm Hg)	92 ± 3	99 ± 2	87 ± 3	95 ± 1
Mean arterial pressure (mm Hg)	111 ± 3	120 ± 2 *	106 ± 3	115 ± 1 *
Pulse pressure (mmHg)	44 ± 2	47 ± 2	44 ± 2	46 ± 2
Heart rate (beats/min)	365 ± 8	377 ± 10	305 ± 5	315 ± 10
Locomotor activity (counts/min)	3.2 ± 0.3	2.2 ± 0.4	0.4 ± 0.1	0.4 ± 0.1

Table 6	. Syster	nic arterial b	lood j	pressure,	heart rate,	and loce	omoto	or activity
during	active	(night-time)	and	resting	(daytime)	periods	in 1	neonatally
hypoxic	and co	ntrol female r	ats at	t two mo	nths of age			

	Active (N	ight-time)	Resting (Daytime)
	Control $(n-7)$	Hypoxic $(n-7)$	Control $(n-7)$	Hypoxic $(n-7)$
Systolic arterial pressure (mm Hg)	(n - r) 123 ± 3	(n - 7) 124 ± 3	(n - r) 116 ± 3	(n - 7) 117 ± 3
Diastolic arterial pressure (mm Hg)	79 ± 2	80 ± 3	77 ± 2	79 ± 1
Mean arterial pressure (mm Hg)	99 ± 2	100 ± 3	92 ± 2	93 ± 3
Pulse pressure (mmHg)	42 ± 1	45 ± 1	43 ± 1	45 ± 1
Heart rate (beats/min)	456 ± 7	442 ± 6	380 ± 9	378 ± 14
Locomotor activity (counts/min)	5.3 ± 0.5	6.5 ± 1.4	1.0 ± 0.1	2.0 ± 0.7

Table 7	. Systen	nic arterial b	lood j	pressure,	heart rate,	, and loco	omot	tor activity
during	active	(night-time)	and	resting	(daytime)	periods	in	neonatally
hypoxic	and co	ntrol female r	ats at	t three m	onths of ag	e		

	Active (N	light-time)	Resting (Daytime)	
	Control	Hypoxic	Control	Hypoxic
	(<i>n</i> = 8)	(<i>n</i> = 8)	(<i>n</i> = 8)	(<i>n</i> = 8)
Systolic arterial pressure (mm Hg)	137 ± 2	133 ± 2	129 ± 2	124 ± 2
Diastolic arterial pressure (mm Hg)	88 ± 2	85 ± 3	80 ± 3	78 ± 3
Mean arterial pressure (mm Hg)	110 ± 2	107 ± 3	102 ± 3	99 ± 3
Pulse pressure (mmHg)	50 ± 1	48 ± 1	49 ± 1	46 ± 1
Heart rate (beats/min)	441 ± 9	415 ± 6	358 ± 8	342 ± 6
Locomotor activity (counts/min)	5.1 ± 0.6	5.3 ± 0.4	1.0 ± 0.1	1.6 ± 0.3

Table 8. Systemic arterial blood pressure, heart rate, and locomotor activity during active (night-time) and resting (daytime) periods in ovairectomized neonatally hypoxic and control female rats at three months of age

	Active (N	ight-time)	Resting (Daytime)					
	Control	Hypoxic	Control	Hypoxic				
	(<i>n</i> = 6)	(<i>n</i> = 6)	(<i>n</i> = 6)	(<i>n</i> = 6)				
Systolic arterial pressure (mm Hg)	129 ± 1	132 ± 2	126 ± 2	126 ± 2				
Diastolic arterial pressure (mm Hg)	87 ± 1	89 ± 2	83 ± 2	83 ± 2				
Mean arterial pressure (mm Hg)	105 ± 1	109 ± 2	102 ± 2	103 ± 2				
Pulse pressure (mmHg)	42 ± 1	43 ± 1	43 ± 1	42 ± 2				
Heart rate (beats/min)	380 ± 6	381 ± 9	329 ± 7	319 ± 7				
Locomotor activity (counts/min)	4.9 ± 0.5	4.9 ± 0.3	1.0 ± 0.1	1.2 ± 0.2				
Table 9	. Systen	nic arterial b	lood j	pressure,	heart rate,	, and loco	omoto	or activity
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during	active	(night-time)	and	resting	(daytime)	periods	in 1	neonatally
hypoxic	and co	ntrol female r	ats at	t six mon	ths of age			

	Active (Night-time)		Resting (Daytime)	
	Control	Hypoxic	Control	Hypoxic
	(n = 12)	(n = 7)	(n = 12)	(n = 7)
Systolic arterial pressure (mm Hg)	134 ± 2	134 ± 3	128 ± 2	128 ± 2
Diastolic arterial pressure (mm Hg)	85 ± 1	86 ± 2	79 ± 1	80 ± 1
Mean arterial pressure (mm Hg)	106 ± 1	107 ± 2	101 ± 1	102 ± 2
Pulse pressure (mmHg)	49 ± 1	48 ± 2	49 ± 1	48 ± 2
Heart rate (beats/min)	409 ± 4	397 ± 5	349 ± 5	331 ± 6 *
Locomotor activity (counts/min)	4.1±0.5	3.2 ± 0.4	0.8 ± 0.1	0.7 ± 0.3

Data are presented as mean value \pm S.E.M. Each value represents the average of 12-h active (night-time) or resting (daytime) periods; $p \le 0.05$ vs. control.

Table 10. Effect size of neonatal hypoxia on active (night-time) systemicarterial pressure values in male rats at two, three, and six months of age.

		95% Confidence Interval		
	Effect size	Lower	Upper	
2 months	0.82	0.00	1.59	
3 months	1.12	0.21	2.02	
6 months	1.78	0.49	3.06	

Table 11. Baroreflex sensitivity (BRS) during active (night-time) and resting(daytime) periods in neonatally hypoxic and control male rats at two, three,and six months of age

	Active (N	Night-time)	Resting (Daytime)		
	Control	Hypoxic	Control	Hypoxic	
2 Month BRS (ms/mmHg)	1.30 ± 0.16	0.93 ± 0.09 *	1.43 ± 0.11	1.27 ± 0.10	
3 Month BRS (ms/mmHg)	1.46 ± 0.15	1.16 ± 0.08	1.89 ± 0.18	1.63 ± 0.11	
6 Month BRS (ms/mmHg)	1.56 ± 0.16	1.48 ± 0.23	2.18 ± 0.24	2.12 ± 0.41	

Data are presented as mean value \pm S.E.M. Each value is calculated from 30 minutes of active (night-time) or resting (daytime) data at noon and midnight; p \leq 0.05 vs. control.

Explanation of Baroreflex Sensitivity Calculation Using the Sequence Method

This example illustrates the baroreflex of concept sensitivity and its calculation using the sequence method. For illustrative purposes, R-Rinterval is used in place of interval. pulse The calculation begins by looking for an increasing or decreasing sequence of systolic blood pressure values. In this example, a sequence of increasing



systolic values is shown in Figure 6. Illustration of how baroreflex sensitivity the middle of Figure 6. is calculated using the sequence method. These points have been (Adopted from Persson, 2001^{1})

labelled SBP 1, 2, and 3. The next step is to look for a proceeding sequence of RR-intervals that change in the same direction as systolic blood pressure, which strongly indicates a baroreflex-mediated response. The top of Figure 6 shows electrical activity of the heart and an increasing sequence of RR intervals. They have been labelled RR 1, 2, and 3 to correspond to their preceding systolic blood pressure values. The relationship between the two is shown in the bottom of Figure 6. Baroreflex sensitivity is obtained by plotting SBP 1, 2, and 3 along the x-axis, measured in mmHg, against the resulting RR 1, 2, and 3 values along the y-axis, measured in ms. The slope created by these points gives an index of baroreflex sensitivity and has the units of ms/mmHg. In reality, many sequences from a single individual are used. Figure 7 is one example of the many sequences

taken from one rat. The y-axis is again measured in ms and the x-axis is once again systolic blood pressure in mmHg. Thirty minutes of systolic blood pressure and pulse interval values were analyzed to determine all sequences with directionally similar changes in both variables. Before baroreflex sensitivity can be determined, the slope for each individual sequence is calculated. Then, the average of all the individual slopes finally gives baroreflex sensitivity.



Figure 7. Baroreflex sequences from one rat over a 30 minute period.

Alveolar Gas Equation

The alveolar gas equation was used to estimate arterial oxygen saturation during neonatal hypoxia. In the rat, hemoglobin is entirely composed of adult globin chains by day 18 of gestation, and similar levels of hypoxia have been shown to decrease arterial oxygen saturation to approximately 50-70% in the rat¹⁵⁶⁻¹⁵⁸.

$$PAO_2 = FIO_2(P_B - P_{H2O}) - PaCO_2\left(FIO_2 + \frac{1 - FIO_2}{R}\right)$$

PAO ₂ : alveolar partial pressure of oxygen	
FIO ₂ : fraction of inspired oxygen	0.12
$(P_B - P_{H20})$: barometric pressure – water vapour pressure	760-47 mmHg
<i>PaCO</i> ₂ : arterial partial pressure of carbon dioxide	40 mmHg
R : respiratory quotient	0.8

 $PAO_2 = 0.12(713 \ mmHg) - 40 \ mmHg \left(0.12 + \frac{1 - 0.12}{0.8} \right) = 37 \ mmHg$



Figure 8. Oxyhemoglobin dissociation curve in several species, including rats (filled squares). Figure taken from Gray¹⁵⁹

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