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INVITED REVIEW

Developmental determinants of cardiac sensitivity to hypoxia[1]

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Abstract: Cardiac sensitivity to oxygen deprivation changes significantly during ontogenetic development. However, the mechanisms for the higher tolerance of the immature heart, possibilities of protection, and the potential impact of perinatal hypoxia on cardiac tolerance to oxygen deprivation in adults have not yet been satisfactorily clarified. The hypoxic tolerance of an isolated rat heart showed a triphasic pattern: significant decrease from postnatal day 1 to 7, followed by increase to the weaning period, and final decline to adulthood. We have observed significant ontogenetic changes in mitochondrial oxidative phosphorylation and mitochondrial membrane potential, as well as in the role of the mitochondrial permeability transition pores in myocardial injury. These results support the hypothesis that cardiac mitochondria are deeply involved in the regulation of cardiac tolerance to oxygen deprivation during ontogenetic development. Ischemic preconditioning failed to increase tolerance to oxygen deprivation in the highly tolerant hearts of newborn rats. Chronic hypoxic exposure during early development may cause in-utero or neonatal programming of several genes that can change the susceptibility of the adult heart to ischemia–reperfusion injury; this effect is sex dependent. These results would have important clinical implications, since cardiac sensitivity in adult patients may be significantly affected by perinatal hypoxia in a sex-dependent manner.

Key words: immature heart, ontogeny, hypoxic tolerance, cardiac protection.

Résumé : La sensibilité cardiaque à la privation d'oxygène change significativement au cours de l'ontogénie. Cependant, les mécanismes responsables de la tolérance plus élevée du cœur immature, ainsi que les possibilités de protection et de l'impact potentiel d'une hypoxie périnatale sur la tolérance cardiaque à la privation d'oxygène chez les adultes n'ont pas encore été clarifiés de manière satisfaisante. La tolérance hypoxique du cœur de rat isolé a révélé un patron triphasique : une diminution significative du jour post-natal 1 au jour 7, suivie d'une augmentation lors du sevrage et d'un déclin final à l'âge adulte. Les auteurs ont observé des changements ontogéniques significatifs sur le plan de la phosphorylation oxydative mitochondriale, du potentiel membranaire mitochondrial, ainsi que du rôle des pores de transition de perméabilité de la mitochondrie dans le dommage myocardique. Ces résultats appuient l'hypothèse que les mitochondries cardiaques sont profondément impliquées dans la régulation de la tolérance cardiaque à la privation d'oxygène durant l'ontogénie. Le pré-conditionnement ischémique ne parvenait pas à augmenter la tolérance à la privation d'oxygène chez les cœurs de rats nouveau-nés, hautement tolérants. L'exposition chronique à l'hypoxie, tôt dans le développement, peut induire la programmation in utero ou néonatale de plusieurs gènes qui peuvent modifier la susceptibilité du cœur adulte au dommage d'ischémie/reperfusion; cet effet est dépendant du sexe. Ces résultats pourraient avoir des implications cliniques importantes, puisque la sensibilité des patients adultes peut être significativement affectée par l'hypoxie périnatale de manière dépendante du sexe. [Traduit par la Rédaction]

Mots-clés : cœur immature, ontogénie, tolérance hypoxique, protection cardiaque.

Introduction

The most frequent (and hence the most widely studied) cardiovascular diseases of modern times undoubtedly include hypoxic states. They originate as a result of disproportion between the amount of oxygen supplied to the cardiac cell and the amount actually required by the cell. Whereas a lot of data are available concerning the effect of hypoxia on the adult myocardium, much less is known about the consequences of oxygen deprivation to the immature heart. Our present interest in the developing heart is driven by clinical urgency: (*i*) ischemic heart disease is no longer the disease of the fifth and older decades; its origin as well as risk factors are already present during early ontogeny, (*ii*) the number of adult patients with ischemic heart disease that were operated for cyanotic congenital heart disease during infancy is steadily increasing. This group of patients is growing older, and is approaching the age characterized by significantly increased risk of serious cardiovascular diseases, such as hypertension and ischemic heart disease. It can be expected that more of such patients will require diagnostic or therapeutic catheterization or cardiac surgery. Under these conditions, the question of the presumed cardiovascular impact of perinatal hypoxia will be of considerable importance. Moreover, the developmental approach can substantially help in the search for the pathogenetic mechanisms involved during the whole ontogenetic development. The aim of this review is, therefore, to summarize current data on the effects of oxygen deprivation on the developing mammalian heart.

Hypoxia versus ischemia

Oxygen, an essential substrate for cell survival, acts as the final electron acceptor in the electron transport chain. In humans,

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oxygen tension varies from 100 mm Hg in alveolar capillaries to between 40 and 20 mm Hg in systemic tissues (1 mm Hg = 133.322 Pa). Theoretically, any of the known mechanisms leading to tissue hypoxia can be responsible for a reduced oxygen supply in the myocardium, but the most common causes are undoubtedly (*i*) stagnant (ischemic) hypoxia, induced by reduction or interruption of the coronary blood flow, and (*ii*) hypoxic (systemic) hypoxia characterized by a decrease in PO_2 in the arterial blood but adequate perfusion. In 2 cases, however, systemic hypoxia can be qualified as physiological: the fetal myocardium that is adapted to hypoxia corresponding to an altitude of 8000 m a.s.l. ("Mount Everest in utero", Eastman 1954) and the myocardium of subjects living permanently at high altitudes. For the sake of completeness, we would add (*iii*) anemic hypoxia in which the arterial PO_2 is normal, but the oxygen transport capacity of the blood is decreased, and (*iv*) histotoxic hypoxia resulting from reduced intracellular utilization of oxygen, e.g., inhibition of oxidative phosphorylation in cyanide poisoning. The most frequent causes for a high oxygen requirement are physical activity, mental stress, or administration of a substance with positive inotropic and chronotropic effects. In healthy subjects, these high oxygen requirements are adequately met by an increase in the coronary blood flow (Ostadal et al. 1999).

It should be emphasized that the terms "hypoxia" and "ischemia" are often used interchangeably in the literature, despite the fact that the consequences of the 2 mechanisms are very different at the cellular level. In ischemia, there is not only a drop in the supply of oxygen and substrates, but also a significant reduction in the clearance of metabolites, in particular of lactic acid and hydrogen ions; the intracellular pH falls rapidly. Systemic hypoxia is usually a generalized phenomenon diffusely involving the whole myocardium, whereas ischemia is confined to the area supplied by the affected coronary artery. Ischemic hypoxia is clinically manifested primarily in ischemic heart disease, whereas systemic hypoxia is associated with chronic cor pulmonale of varying origin, cyanosis due to a hypoxemic congenital heart disease, and changes in the cardiopulmonary system induced by a decrease in barometric pressure at high altitudes (Ostadal and Kolar 1989).

With a reduction in oxygen supply, the adult as well as the immature myocardium switches from the aerobic to the anaerobic mode, and the capacity of the myocytes to generate energy in the form of ATP and creatine phosphate becomes severely reduced (Lopaschuk et al. 1992). Energy depletion, oxygen radical accumulation, loss of calcium homeostasis, and loss of osmotic control lead to contractile dysfunction, membrane disruption, and finally to death of cardiac cells. The degree of myocardial injury depends not only on the intensity and duration of hypoxic (ischemic) stimulus but also on the degree of cardiac sensitivity to oxygen deficiency. This particular parameter changes significantly during postnatal ontogeny. This is not surprising because most of the determinants of the relationship between myocardial oxygen supply and demand change markedly during development.

Hypoxia and the fetal mammalian heart

Physiological hypoxia (as compared with the arterial PO_2 in the mother) is a normal part of fetal life for all vertebrates and has a significant role in vasculogenesis, angiogenesis, hematopoiesis, and chondrogenesis during fetal development (Ream et al. 2008, for a review see Sedmera and Ostadal 2012). The partial oxygen tension of a developing embryo is <10 mm Hg, which is regarded as being hypoxic compared with normal tissue with an oxygen tension of 20–40 mm Hg (Webster and Abela 2007). This suggests that the fetus is persistently hypoxic during organ formation, growth, and maturation, and that fetal tissues have a lower threshold at which they reach a state of oxygen deprivation (Patterson and Zhang 2010). It is necessary to mention that fetal hemoglobin with the leftward shift of the oxygen dissociation curve plays an important role in coping with tissue hypoxia during prenatal life. The expression of hypoxia-induced genes, such as *hypoxia inducible factor 1* (*HIF 1*) and *vascular endothelial growth factor* (*VEGF*), correlate with angiogenesis, vasculogenesis, and heart remodeling (Tomanek et al. 1999; Compernolle et al. 2003; Sugishita et al. 2004). Moreover, Yue and Tomanek (1999) have demonstrated that hypoxia is the major stimulus for vessel growth during fetal development. Interestingly, the fetal heart is more tolerant to hypoxia-induced cell death than the adult heart, owing to, among others, its enhanced ability to increase glycolytic flux (Ascuitto and Ross-Ascuitto 1996).

Although fetal hearts show a remarkable ability to survive and function under low oxygen tension (Sedmera et al. 2002), chronically pathological hypoxia is associated with numerous complications that have both short- and long-term (i.e., persisting till adulthood) effects (for a review see Patterson and Zhang 2010). The fetus may experience prolonged hypoxic stress under many different conditions, including pregnancy at high altitude, pregnancy with anemia, placental insufficiency, cord compression, and heart, lung, and kidney disease. There is clear evidence of a link between hypoxia and fetal intrauterine growth restriction. Human studies at high altitude suggest that hypoxia per se, independent of maternal nutrition, causes fetal growth restriction, resulting in low birth weight and altered body shape at birth (Giussani et al. 2001).

Experimental studies have demonstrated that reduced fetal oxygen supply causes incomplete development of the heart, like ventricular septal defects, myocardial thinning, ventricular dilatation, and epicardium detachment, and slows fetal heart maturation (Sharma et al. 2006; Ream et al. 2008; Nanka et al. 2008). In fetal sheep, long-term hypoxemia (natural altitude 3820 m a.s.l., 110 days) reduced cardiac output and contractility (Gilbert 1998), increased lactate dehydrogenase and citrate synthase (Ohtsuka and Gilbert 1995), and resulted in cardiomegaly (Murotsuki et al. 1997; Martin et al. 1998). In addition to cardiomyocyte hypertrophy, alterations in components of the extracellular matrix (ECM), specifically interstitial collagens in the heart, are seen in cardiac remodeling caused by hypoxia. Meanwhile, the changes in matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) might be initiated to compensate for the accumulation and deposition of collagens; however, the interruption of the fine balance between MMPs and TIMPs after hypoxia might eventually decompensate and impair the fetal heart morphology and function (Tong and Zhang, 2011).

Bae et al. (2003) have observed that maternal chronic hypoxia (10.5% oxygen, 7 days) led to the expression of HIF1α and increased apoptotic cell death in fetal rat hearts. The increased cell death may induce asymmetric cardiac enlargement. Although the mechanisms underlying hypoxia-induced apoptosis are not clear and are likely to be complex, the above study demonstrated that apoptosis was associated with an increase in Fas receptors and decrease in Bcl-2 proteins. In addition, chronic hypoxia significantly suppressed the expression of heat shock protein (Hsp) 70 and differentially regulated β-adrenoreceptor (AR) subtypes in the fetal heart. There was no difference in β_2-AR protein levels between the control and hypoxic heart, but β_1-AR protein levels were significantly increased in the hypoxic group. Recently, Patterson et al. (2010) have observed that hypoxic treatment of pregnant rats from days 15 to 21 of gestation resulted in a significant decrease in PKCε protein and mRNA levels in fetal hearts. This study demonstrated a direct effect of hypoxia on epigenetic modification of DNA methylation and programming of cardiac *PKCε* gene repression in a sex-dependent manner, linking fetal hypoxia to possible pathophysiological consequences in the hearts of adult offspring, as PKCε is known to play a pivotal role in cardioprotection against ischemia–reperfusion (I/R) injury.

A population-based, prospective cohort study has investigated fetal hemodynamic adaptive changes related to intrauterine growth retardation (Verburg et al. 2008). Fetal growth characteristics and fetal circulation variables were assessed with ultrasound and Doppler examinations in 1215 healthy women. It was observed that decreased fetal growth is associated with cardiac remodeling and changes of cardiac output, consistent with a gradual increase in afterload and compromised arterial compliance. These changes have already begun to occur before the stage of clinically apparent fetal growth restriction.

These data imply that limitation of either oxygen or nutrient supply to the fetus produces functional and structural changes in the neonatal heart. However, less is known regarding the persistence and long-term consequences of these changes and their possible contribution to the increased risk of cardiovascular disease in later life (see below).

Cardiac hypoxia during postnatal development

The mammalian organism is not fully developed at birth, and the process of maturation thus continues during the immediate postnatal period (Patel and Srinivasan 2010). It is evident that this period of immaturity is characterized by a great plasticity, with critical windows of opportunity during which any adequate insult or intervention may positively or adversely influence postnatal growth and development. According to this theory, the tissues are most sensitive to injury during the period of intensive growth (Ostadalova and Babicky 2012). The precise knowledge of individual ontogenetic periods critical for cardiac ontogeny is thus crucial for the prediction and explanation of cardiac reactions to various pathogenetic stimuli (Fig. 1).

Cardiac tolerance of the immature heart to acute oxygen deficiency is significantly higher compared with the adult myocardium. Riva and Hearse (1993) observed that age-dependent changes in resistance to global ischemia in the isolated rat heart showed a biphasic pattern with increasing tolerance from 5 to 23 days of age, followed by a decline to adulthood. Detailed analysis of the tolerance of the isolated rat heart to global ischemia in the course of the first week of life has revealed a significant decrease in recovery of the developed force from day 1 to 7 (Fig. 2; Ostadalova et al. 1998; Riva and Hearse 1993), suggesting a possible triphasic pattern of the ontogenetic development of cardiac sensitivity to ischemia. The developmental changes are sex-dependent: cardiac tolerance was similar in males and females up to the end of the weaning period; however, it decreased in males from the 30th to the 60th day, but remained unchanged in females. The adult female heart is thus significantly more resistant to oxygen deprivation than the male heart (for a review see Ostadal et al. 2009*a*; Ostadal and Ostadal 2014).

In this connection it is interesting to mention that, at least in rats, body growth is also not linear during the early postnatal period (Ostadalova and Babicky 2012). There are 2 small periods of retardations: the first week of life, and the weaning period. The decline during the first days of postnatal life is evidently connected with dramatic changes at birth (see later) and slowly appearing maternal milk production. Slowdown of the body growth around the 16th day of postnatal life (beginning of the weaning period) can be explained by relatively insufficient caloric supply from the milk; this also stimulates the transition to solid food (Babicky et al. 1970). It seems, therefore, that increase in cardiac tolerance to hypoxia during the early phases of ontogeny is somehow related to the decrease in body growth.

The mechanisms for the higher resistance of the neonatal heart to oxygen deprivation have not yet been satisfactorily clarified (for a review see Ostadal et al. 2009*b*). For the explanation of this fact, the physiological alterations during the perinatal period should be taken into consideration. The major changes in oxygen saturation can be observed within delivery: during the short

Fig. 1. Critical periods in the early postnatal development of rats. Adapted from Ostadalova and Babicky (2012).

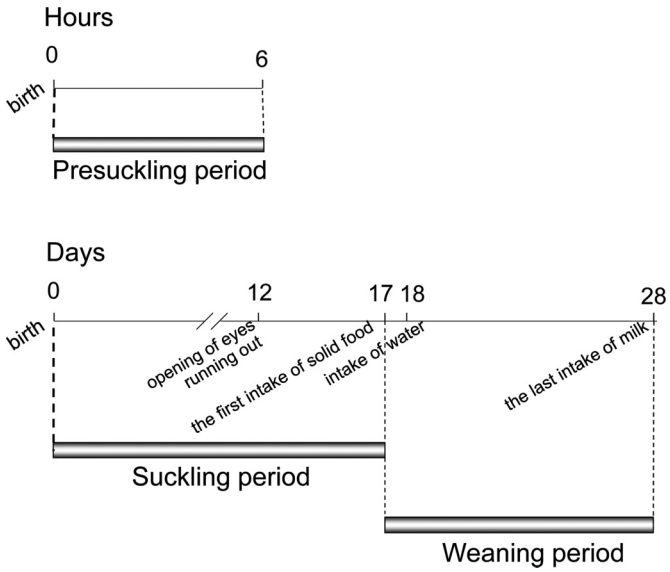
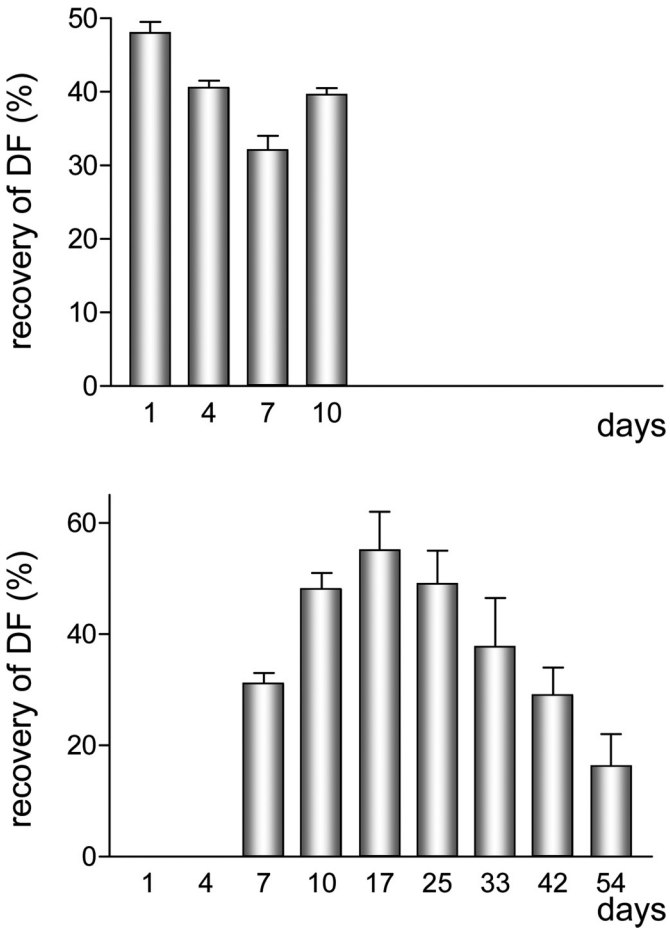


Fig. 2. Tolerance of the isolated perfused rat heart to acute ischemia–reperfusion (expressed as the recovery of the developed force (DF)). Data for days 1–10 from Ostadalova et al. (1998); data for days 7–54 from Riva and Hearse (1993).



period of time that the mammalian fetus (and its heart) comes from the hypoxic environment with low PO_2 and low oxygen saturation (18%) into the normal atmosphere (PO_2 160 mm Hg), arterial saturation increases more than 5 times (to 97%). The delivery is, furthermore, accompanied by the transition from the amniotic fluid to the air, by the marked decrease of ambient temperature, by the termination of placental nutrition, and by oxidative stress. This transition requires appropriate physiological adaptations: onset of pulmonary respiration, transition from fetal to neonatal circulation, switching-on of thermoregulation, and increase of basal metabolic rate. As a consequence of the dramatic changes at birth, mammalian hearts suddenly meet an extremely high concentration of reactive oxygen species (ROS). The neonatal heart can probably use ROS for the upregulation of protein degradation that permits the production of amino acids, which are necessary for the maintenance of energy homeostasis during neonatal starvation (Kuma et al. 2004; Mühlfeld et al. 2005; Ostadalova et al. 2010).

The reason of the higher tolerance during further ontogenetic development can be still only hypothetical (for a review see Ostadal et al. 2009*b*; Sedmera and Ostadal 2012). It may be speculated that an explanation of the phenomenon lies in the greater anaerobic glycolytic capacity, higher glycogen reserves of the immature heart (Hoerter 1976), amino acid utilization by transamination (Julia et al. 1990), and changes in calcium handling (Vetter et al. 1995; Nijjar and Dhalla 1997). It is interesting to note that calcium overload, common in the adult myocardium, was not described in the immature heart.

Recently, Liaw et al. (2013) demonstrated that neonatal hearts exhibit greater Akt reserves available for phospho-activation compared with mature hearts. Downstream at one of the major effectors, neonatal hearts show the greatest degree of phospho-inhibition of glycogen synthase kinase 3β. Moreover, neonatal tissue exhibits high baseline expression of survival proteins HIF1α and Cav-3, autophagic proteins LC3B and Beclin1, and apoptotic regulators Bax and Bcl-2. In terms of postnatal changes of myocardial tolerance to oxygen deprivation, these data reveal a complex series of changes in pro-survival and pro-death proteins with maturation. None of the above results can, however, fully explain the day-by-day changes in cardiac tolerance to oxygen deprivation during the first week of life. Our previous observations have shown that the early postnatal development of cardiac contractile function and its regulation at the level of Ca^{2+} transport exhibited significant day-by-day changes in the first week of life (Ostadalova et al. 1993, 1995). The changes are due to a disproportion between the rapidly increasing functional demands and the structural and functional ability to fulfill these requirements.

The role of mitochondria in the developmental changes of cardiac tolerance to oxygen deprivation is still unclear, in spite of the fact that mitochondria are responsible for cellular oxygen handling. Mitochondrial oxidative phosphorylation is not completely developed in the rat heart at birth; cardiac maturation during the first postnatal week is characterized by increasing content and specific activity of cytochrome *c* oxidase and enhanced flux of adenine nucleotides across the inner mitochondrial membrane (Schägger et al. 1995; Drahota et al. 2004). We have shown previously (Skarka et al. 2003) that the content of cytochromes in the rat cardiac mitochondria increased 2-fold between birth and day 30, similar to the expression of adenine nucleotide translocase 1. Moreover, in newborn animals, a single population of mitochondria with relatively high mitochondrial membrane potential was observed. Starting with the weaning period, a second population with significantly lower membrane potential occurs. The collapse of membrane potential owing to the opening of a high conductance mitochondrial permeability transition pore (MPTP) has been implicated in the molecular mechanisms associated with I/R injury of the adult heart (Di Lisa and Bernardi 1998, 2006). We have observed, however, significant ontogenetic differences in the role of MPTP in I/R injury. Whereas the blockade of MPTP by sanglifehrin in perfused rat heart had a protective effect on I/R-induced damage in the adult myocardium, as has already been demonstrated (Di Lisa et al. 2001), it had no effect in the neonatal heart (Fig. 3; Milerova et al. 2010). For the explanation of this difference the possible lower sensitivity of MPTP in the neonatal heart to pore-opening factors has to be taken into consideration. Indeed, we have found (Milerova et al. 2010) that in cardiac mitochondria isolated from neonatal rats, Ca^{2+}-dependent and cyclosporine-sensitive MPTP is less sensitive to Ca^{2+} ions as compared with adults (Fig. 4). We can only speculate that its lower sensitivity to the calcium-induced swelling is related to the higher ischemic tolerance of the neonatal heart. All of these results support the hypothesis that cardiac mitochondria are deeply involved in the regulation of cardiac tolerance to oxygen deprivation during ontogenetic development.

Chronic hypoxia (CH) is the main pathophysiological feature of hypoxemic congenital heart disease. Understanding the mechanisms by which cyanotic congenital heart malformations modify the myocardium and how the modifications impact the cardiac tolerance to oxygen deprivation may provide insight into developing treatments for limiting myocardial damage. Unfortunately, no existing model adequately reproduces chronic myocardial perfusion with the hypoxic blood caused by congenital cyanotic defects; therefore, similar experimental models as in adults, i.e., CH simulated in the normobaric or hypobaric chambers, are used in studies performed during early stages of ontogenetic development. In the chronically hypoxic newborn mammals, body growth is blunted (Chvojkova et al. 2005). Neonatal growth retardation during moderate (15% O_2) or severe (10% O_2) hypoxic exposure can be almost entirely attributed to the effects of hypoxia on the newborn, and is not mediated by the maternal response (Mortola et al. 1990). Faulty maternal lactation and limited food availability to the suckling are not the primary mechanisms for the neonatal growth retardation in chronic hypoxia, as was suggested by the observations that the cellular responses to hypoxia differ from those of experimental starvation (Naye 1966). Mild (19% O_2) or moderate levels of 1-week hypoxic exposure significantly increased cardiac mass and DNA synthesis (Mortola et al. 1990). This suggests that hypoxia can truly stimulate cardiac muscle cell multiplication, as it has been demonstrated in neonatal rats exposed to sideropenic anemia (Rakusan and Poupa 1966; Neffgen and Korecky 1972), or to low oxygen atmosphere (Hollenberg et al. 1976; Wachtlová et al. 1977). In neonatal animals exposed to high altitude, the cardiac enlargement and activation of DNA synthesis was significantly more expressed in the right ventricular myocardium. The mechanisms behind the hypoxia-induced cardiac hyperplasia are unclear, but it is possible that they relate to the greater cardiac work caused by the higher cardiac output, blood viscosity, and pulmonary vascular resistance.

Cardiac protection of the immature heart

As mentioned above, cardiac tolerance of the immature heart to oxygen deficiency is significantly higher as compared with the adult myocardium. Thus the question arises whether we can further increase the already high resistance of the immature mammalian heart. To the most effective experimental protective mechanisms belong long-lasting adaptation to CH (for a review see Ostadal and Kolar 2007) and various forms of conditioning (for a review see Bolli 2007). Whereas abundant data are available on these 2 phenomena in adults, the information on the immature heart are, however, only sporadic. Data on the possible effect of postconditioning on the immature heart are still lacking (for a review see Ostadal et al. 2009*b*).

We have shown (Ostadalova et al. 1998) that classical ischemic preconditioning (IP), at least in rats, is not present at birth, and that the enhanced postischemic recovery of contractile function

Fig. 3. The effect of sanglifehrin A (inhibitor of mitochondrial permeability transition) on lactate dehydrogenase (LDH) content after ischemia–reperfusion injury in the hearts from neonatal (7-day-old) and adult rats. Data are the average values from 17 neonatal and 14 adult rats; *, $p < 0.01$ when comparing both age groups. Data from Milerova et al. (2010).

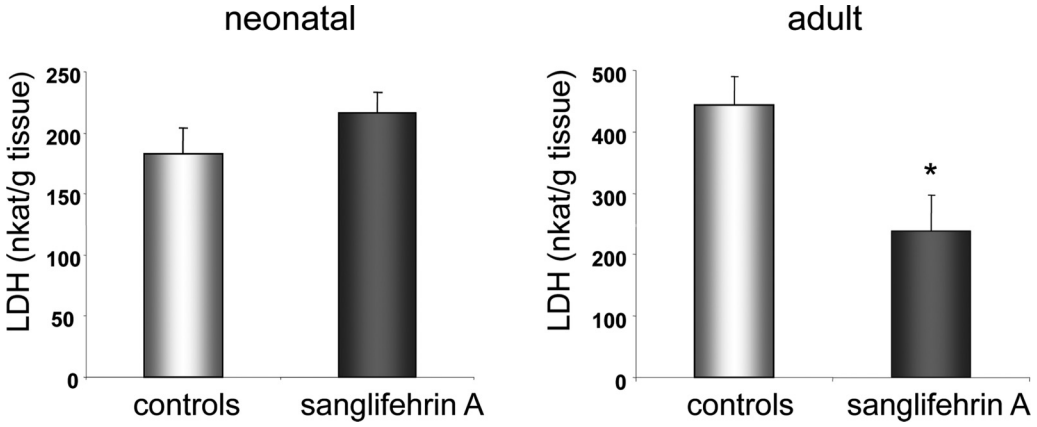
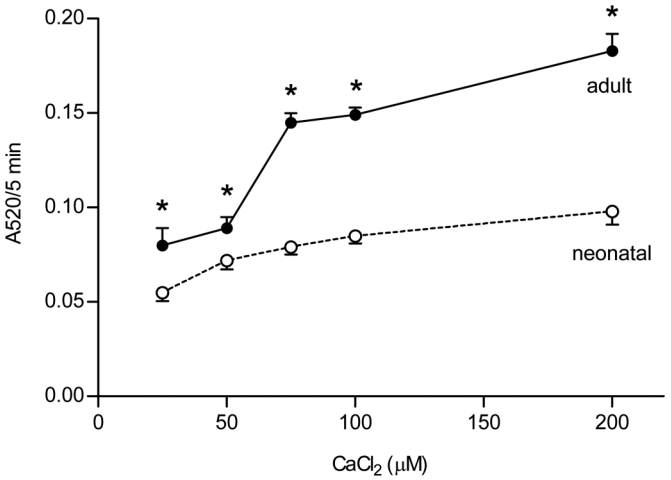


Fig. 4. The extent of mitochondrial swelling of cardiac mitochondria (determined as the decrease in absorbance at 520 nm) from adult and neonatal (7-day-old) rats; *, $p < 0.01$ when comparing both age groups. Reproduced with permission from Ostadal et al. (2009*b*).

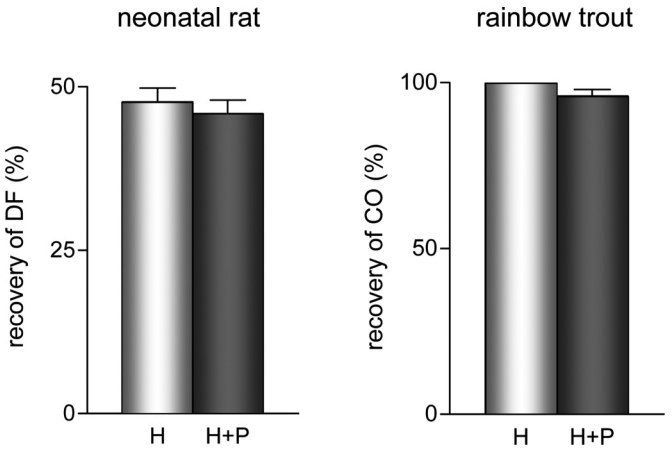


can be observed only at the end of the first postnatal week. The decreasing tolerance of the neonatal heart to ischemia is thus counteracted by the development of the inducible endogenous protective mechanism. Molecular mechanisms of IP in the adult myocardium are still unclear, and the same is true for the immature heart. Signaling cascade obviously starts by the release of some "protective molecules". For example the activation of the PI3K–Akt pathway as part of the Reperfusion Injury Salvage Kinase (RISK) pathway (Hausenloy and Yellon 2007; Hausenloy et al. 2012) at the onset of myocardial reperfusion has been reported to underlie the cardioprotection elicited by different types of myocardial conditioning (Yellon and Downey 2003; Hausenloy et al. 2005). The end-effector of this cascade might be, e.g., the mitochondrial ATP-dependent potassium channels (Garlid et al. 2003). Baker et al. (1999) have observed that activation of mitochondrial K_{ATP} channels is associated with IP even in the immature rabbit heart. On the other hand, in the immature rat heart the administration of 5-hydroxydecanoate, a selective blocker of the mitochondrial K_{ATP} channels, had no effect on the protection by IP; similar results were obtained with the nitric oxide synthase inhibitor (L-NAME) (Ostadalova et al. 2002). It seems, therefore, that there may exist significant interspecies differences in the mechanism of IP in the immature heart. It remains unanswered whether the mechanisms for IP participating in the immature heart differ from the mechanisms described for the adult myocardium.

Already in the late 1950s, the first observation appeared (Hurtado 1960) showing that the incidence of myocardial infarction is lower in people living at high altitude. These epidemiological studies on the protective effect of high altitude were confirmed in experiments using simulated hypoxia (for a review see Ostadal and Kolar 2007). However, only a few authors have compared tolerance to oxygen deprivation in the chronically hypoxic versus normoxic immature myocardium. We have observed (Ostadal et al. 1995) that CH, simulated in the hypobaric chamber, results in similarly enhanced cardiac resistance in rats exposed to CH either from the fourth day of postnatal life or in adulthood. Similarly, Baker et al. (1995) demonstrated that adaptation to CH increased the tolerance of the developing rabbit heart. However, it follows from our results (Ostadalova et al. 2002) that the protective effect of CH is absent in newborn rats and the protective effect of adaptation develops, similarly to IP, only during the first postnatal week. These results suggest that we might be dealing with the more general biological phenomenon: the already high resistance of the cardiac muscle cannot be further increased by another protective mechanism. A similar situation as in the immature mammalian heart can also be observed in the highly tolerant hearts of poikilotherms (Fig. 5; Overgaard et al. 2004) or in the myocardium of young-adult females (for a review see Ostadal et al. 2009*b*). Other adaptive responses to CH, including polycythemia, hypoxic pulmonary hypertension, and right ventricular enlargement, are comparable in animals adapted from birth or adulthood (Ostadal and Kolar 2007).

The overwhelming majority of studies analyzing the possible protective mechanisms of cardiac adaptation to CH deal exclusively with the adult myocardium. Moreover, this phenomenon has been much less studied than in IP, and the understanding of its mechanism is thus still very limited Nevertheless, it seems that various protective phenomena, including both short-lived IP and long-lasting effects of CH, utilize essentially the same endogenous pool of protective pathways, even with different efficiency. They include K_{ATP}, ROS, NO, different protein kinases, opioids, and erythropoietin; however, other factors cannot be excluded (for a review see Kolar and Ostadal 2004). Baker et al. (1998) and Shi et al. (2000) found that the exposure of immature rabbit hearts to CH increased protein levels for endothelial NO synthase as well as release of nitrite, nitrate, and tissue cGMP content. NO synthase inhibitors abolished the cardioprotective effect of hypoxia in 7- and 10-day-old rabbits. We have shown previously (Ostadalova et al. 2002) that the blockade of NO synthase with L-NAME or the blockade of mitochondrial K_{ATP} channels with 5-hydroxydecanoate

Fig. 5. Effect of preconditioning on the 1-day-old rat heart (left, expressed as the recovery of the developed force (DF); data reproduced from Ostadalova et al. (1998)) and the heart of rainbow trout (*Oncorhynchus mykiss*) (right, expressed as the recovery of cardiac output (CO); H, hypoxia; H+P, hypoxia and preconditioning; data from Overgaard et al. (2004)).



completely abolished the cardioprotective effect of adaptation to CH also in 10-day-old rats. It seems, therefore, that unlike in IP, both mitochondrial K_{ATP} channels and NO may play an important role in the mechanisms of adaptation of the neonatal heart to CH. And how might NO interact with mitochondrial K_{ATP} channels? Baker et al. (2001) proposed that NO leads to activation of K_{ATP} channels via soluble guanylyl cyclase, causing the accumulation of cGMP and activation of cGMP-dependent protein kinase. Moreover, Sasaki et al. (2000) demonstrated that exposure of myocytes to an NO donor directly activates mitochondrial K_{ATP} channels. Furthermore, Rakusan et al. (2007) have observed that angiotensin II is also involved in the mechanisms of adaptation of the immature heart to CH: the chronic blockade of angiotensin II type 1 receptors by irbesartan surprisingly, in contrast to adults, completely abolished the cardioprotective effect of CH. These observations should be taken into consideration in the treatment of children suffering from cyanotic congenital heart disease.

As it has been mentioned above, CH is the main pathophysiological feature of hypoxemic congenital heart disease. The timing of corrective surgery is critically important, with early surgery desirable to promote more normal development. Many children undergoing cardiac surgery in the first year of life exhibit varying degrees of cyanotic heart disease where the myocardium is chronically perfused with hypoxic blood. We have observed (Samanek et al. 1989) metabolic adaptation to chronic hypoxia in the myocardium of children with cyanotic congenital cardiac malformations. The aerobic capacity of the energy metabolism was significantly reduced in hypoxic hearts as compared with normoxic patients. Understanding the mechanisms by which cyanotic congenital heart disease modifies the myocardium and how that modifications impact on the cardiac tolerance to ischemia may provide insight into the developing treatments for limiting myocardial damage during cardiac surgery (Fitzpatrick et al. 2005). Unfortunately, the clinical data are still lacking.

Impact of perinatal chronic hypoxia on cardiac tolerance to ischemia in adults

Human epidemiological studies have shown a clear association between adverse intrauterine environment and an increased risk of ischemic heart disease in later adult life (Barker et al. 1989; Barker 2000). One of the most common insults during perinatal development is hypoxemia due to congenital cyanotic heart defects or pulmonary disease secondary to prematurity. Such hypoxemia may persist for several weeks or months until surgical repair of the structural defects or improvement in pulmonary function makes the individual normoxemic (Rohlicek et al. 2002).

Experimental studies on the late effects of CH on cardiac tolerance to hypoxia and ischemia are unfortunately not concise; moreover, most of them have used exclusively males. Furthermore, they differ in the critical ontogenetic period studied (prenatal, perinatal, early postnatal), as well as the intensity and duration of hypoxia (for a review see Ostadal et al. 2011). Nevertheless, animal studies have repeatedly suggested a possible link between early hypoxia and increased risk of cardiovascular disease in offspring (e.g., Li et al. 2003; Netuka et al. 2006; Rueda-Clausen et al. 2011). Li et al. (2003) have found that prenatal CH significantly increases the sensitivity of the adult 6-month-old rat heart to I/R injury, as indicated by increased myocardial infarct size and decreased postischemic recovery of left ventricular function. However, in their later study on 2-month-old animals (Li et al. 2003), I/R caused a comparable degree of infarction in prenatal hypoxic and control rats. We have observed myocardial ischemic injury — as judged from the number of ischemic arrhythmias and increased LDH concentration — already in 3-month-old rats (Netuka et al. 2006). These contradictory results suggest possible age-dependent changes in cardiac tolerance to oxygen deprivation induced by early exposure to chronic hypoxia. Another explanation may be the different duration of ischemia; prolongation may mask the potential differences in ischemic injury. Xu et al. (2006) have demonstrated that prenatal hypoxia (12% oxygen from the day 15 of pregnancy) in 4-month-old rats induced left and right ventricular enlargement, increased expression of collagen I and III, increased ratio of β- to α-myosin heavy chain proteins, and decreased matrix metalloproteinase activity. Cardiac remodeling was consistent with the diastolic dysfunction and increased sensitivity to I/R injury. According to Peyronnet et al. (2002), perinatal exposure of rats to hypoxia exerts adverse effects on the development of the autonomic nervous system related to cardiovascular events and increased hemodynamic response under stress conditions in adults. Furthermore, Rohlicek et al. (2002) observed that adult rats made hypoxemic neonatally showed a markedly greater cardiac output increase during acute hypoxia than the controls. This may be due to altered myocardial function and (or) changes in the autonomic nervous system response to acute hypoxemia. A recent study (Rueda-Clausen et al. 2012) has suggested that the increased susceptibility to myocardial ischemia in prenatally hypoxic animals can be exacerbated by a secondary insult like high-fat diet during the early postnatal period. In this connection it is of interest to mention the results of Hampl and Herget (1990) and Hampl et al. (2003), which showed that perinatal hypoxia also increases the susceptibility to hypoxic pulmonary hypertension later in life.

The mechanisms of the increased susceptibility of the adult hearts to I/R injury in animals exposed to hypoxia during perinatal period are not known at present. Li et al. (2003) have observed that I/R-induced apoptosis was 44% higher in the hearts of adult rats that had experienced prenatal CH compared with the control animals. Consistent with the increased apoptosis, the ratio of cleaved form to pro-form of caspase-3 was significantly higher in hypoxic hearts after I/R, indicating increased caspase-3 activity. Taken together, these results suggest that prenatal CH sensitizes the apoptosis pathway in the adult heart in response to I/R stimulation. In addition, Li et al. (2004) have found that cardiac Hsp 70 and PKCε expression were significantly lower in prenatally hypoxic hearts than in the controls. It has been well-documented that Hsp 70 and PKCε play an important role in protection against I/R injury (Snoeckx et al. 2001; Saurin et al. 2002; Kolar et al. 2009). Their decreased levels may, therefore, play a key role in the increased susceptibility of the adult heart to I/R injury in prenatally hypoxic animals. The finding of decreased eNOS levels in adult prenatally hypoxic hearts suggests an additional potential mech-

anism contributing to their increased susceptibility to I/R injury. These studies suggest that chronic hypoxic exposure during early development may cause in-utero or neonatal programming of several genes that may play an important role in the increased susceptibility of the adult male heart to I/R injury; this programming predisposes the fetus to cardiovascular disease later in life. Maternal cocaine exposure (Bae et al. 2005; Meyer et al. 2009) or maternal vitamin D deficiency (Gezmish and Black 2013) have a similar potential to program long-term vulnerability to cardiovascular disease as perinatal hypoxia.

Clinical and experimental studies suggest that there may be sex-related differences in the cardiac tolerance to ischemia (for a review see Ostadal et al. 2009*a*; Ostadal and Ostadal 2012). The hearts of adult males usually have a higher susceptibility to myocardial injury than the hearts of females. In this connection, the question arises as to whether the effects of perinatal hypoxia on cardiac tolerance to ischemia differ in adult males and females. We have observed in the rat model that the late myocardial effects of hypoxemia, experienced in early life, may be sex-dependent. Perinatal exposure to CH significantly increased cardiac tolerance to acute ischemic injury in adult females, expressed as the lower incidence of ischemic arrhythmias; the effect on arrhythmias in males was the opposite (Netuka et al. 2006). Similar sex-dependent effects from early hypoxia were later confirmed by Xue and Zhang (2009). They have observed that prenatal hypoxia significantly decreased postischemic recovery of left ventricular function, increased cardiac enzyme release, and infarct size in adult male but not female rats.

Recently, 2 studies have analyzed possible mechanisms involved in prenatal hypoxia-induced sex-dependent changes in cardiac tolerance to ischemia in adults. According to Xue and Zhang (2009), these changes are due to differences in fetal programming of *PKCε* gene expression; down-regulation of *PKCε* function was observed in the hearts of adult male offspring only. Patterson et al. (2010) have found that CH during gestation down-regulated *PKCε* expression in the developing heart through an epigenetic modification. They have found sex-related differences in the methylation of specificity protein (SP) 1 binding sites and *PKCε* transcription. Hypoxia-induced methylation was significantly greater in the heart of male fetuses. According to Patterson et al. (2010), this sex difference may be caused, in part, by the greater expression of estrogen receptors α and β in the heart of female fetuses. The finding that both estrogen receptors α and β interacted with the SP1 binding sites at the PKCε promoter in the fetal heart suggests a possible mechanism for the increased protection of SP1 binding sites and *PKCε* transcription in the female hearts in response to hypoxic stress. Although it may be difficult to translate the present findings directly to humans, the possibility that fetal hypoxia may result in sex-dependent programming of a specific gene in the offspring with the consequence of increased cardiac susceptibility to I/R provides an experimental explanation worthy of investigation in humans.

Conclusions

Cardiac sensitivity to oxygen deprivation changes significantly during ontogenetic development. Hypoxic tolerance of the isolated rat heart exhibits a triphasic pattern: significant decrease from postnatal day 1 to 7, followed by increase to the weaning period, and final decline to adulthood. We have observed significant ontogenetic changes in mitochondrial oxidative phosphorylation and mitochondrial membrane potential, as well as in the role of MPTP in myocardial injury. These results support the hypothesis that cardiac mitochondria are deeply involved in the regulation of cardiac tolerance to oxygen deprivation during ontogenetic development. Protective phenomena such as adaptation to chronic hypoxia and ischemic preconditioning failed to increase tolerance to oxygen deprivation in the highly tolerant hearts of newborn rat. Chronic hypoxic exposure during early development may cause in utero or neonatal programming of genes that can change the susceptibility of the adult heart to I/R injury; this effect is sex dependent. These results would have important clinical implications, since cardiac sensitivity in adult patients may be significantly affected by perinatal hypoxia in a sex-dependent manner.

The importance of the developmental approach for experimental and clinical cardiology is indisputable. It offers new possibilities in studies of pathogenesis, as well as the prevention and therapy of serious cardiovascular diseases. Retrieval of developmental mechanisms participating in changes in cardiac tolerance to hypoxia is the best example for this view. It is, however, necessary to state that the interest of developmental cardiologists concentrates almost exclusively on the prenatal development; postnatal changes remain, unfortunately, neglected. Developmental cardiology is now in the molecular era, and new knowledge on the development of the cardiac muscle extends exponentially. Nevertheless, molecular analysis in developmental cardiology is unthinkable without a comprehensive and well-integrated view of the field.

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