Cardiovascular phenotyping in mice

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Abstract

Progress in molecular genetics has changed cardiovascular research. The mouse has turned out to be an invaluable model for mammalian genetic modifications to mimic and analyse cardiovascular pathology. Through the introduction of transgene and gene targeting technology, regulatory systems can be studied at the molecular level. Recent technical developments have downsized the equipment for physiological measurements to the mouse level. Micro-surgery has developed to the level where most manipulations previously performed in larger animals can now be applied to mice. However, different investigators report considerable differences in values for physiological parameters. Whether these differences are related to the variation in mouse strains or experimental procedures remains to be established, but awareness of the variation can be relevant for prospective mouse investigators. In the present review, the physiological measurements performed in mice to date are discussed and complemented with results from genetically manipulated animals. In addition the various surgical procedures and their practical application are illustrated.

Keywords: Phenotype; Mouse; Gene targeting; Hemodynamics; Morphology

1. Introduction

Cardiovascular research is implementing the mouse model for analysis of genetic modifications at a rapid pace [1,2]. There are several reasons for the growing interest to analyse the mouse cardiovascular system. First of all the mouse turned out to be a species in which transgenic and gene targeting experiments could be performed with relative ease. Manipulation of mouse embryonic stem cells has proven to be a reproducible method with a high success rate [3,4]. The expanding knowledge of the mouse genome is another reason for the recent boost in mouse cardiovascular research. Finally, a practical advantage of the mouse is the short gestation period and the low cost of breeding and housing compared to other mammalian models.

Several groups have focused on miniaturising physiology to the mouse level [5,6]. To date it is possible to obtain functional data from the mouse circulation both ex vivo and in vivo [7]. Electrical and hemodynamic parameters can be obtained from intact genetically altered mice, and in addition various pathologic conditions mimicking human cardiovascular disease can be induced by microsurgery [2]. Since a primary goal of mouse research is...
its application to human physiology and disease, it is important to consider the range and limitations of physiological studies in the mouse, as well as the ways in which mouse cardiovascular physiology differs from that of humans. The principal difference, obviously, is physical size. However, other differences in cardiovascular physiology may exist, although they have not been studied systematically. In the present review, the miniaturised methods developed to analyse the cardiovascular phenotype and the structural and functional baseline data obtained in normal mice are discussed. In addition the effect of genetic modification of the mouse on cardiovascular function will be addressed. Transgenesis and gene targeting have been applied extensively to the mouse. To illustrate the synergistic effect of various strategies to unravel physiologic systems involved in cardiovascular control, we review data on the outcome of manipulating the renin–angiotensin system, myocyte calcium handling and adrenergic receptors. To unmask phenotypical changes, interventions to challenge the cardiovascular system may be required. Therefore different surgical techniques adapted to the mouse level are discussed. Data from literature are complemented by preliminary results from mouse studies performed in our laboratory.

2. Structure

2.1. Cardiac anatomy

Although there are some major differences in the morphology of the heart between genera, the differences within mammalian species are only subtle. No differences were found between the cardiac structure of Rb2H/Rb7BmrXC57BL/6d foetuses and C57BL/6JXCBA F1 foetuses [8]. In terms of ventricular structure, the mouse heart is similar to the human heart, with the arrangement of valves providing the best discriminator between the morphological right and left ventricle. In contrast to the human heart, there is little distinction between the apical trabeculations of the left and right ventricle in mice. The most important differences in cardiac structure of man and mouse are confined to the atrial and venous parts. In the mouse heart, unlike the human heart, the left superior caval vein persists and drains into the right atrium. The pulmonary vein has a solitary opening in the left atrium. Moreover there is no extensive formation of a secondary atrial septum [8].

Also the anatomy of the conduction system is somewhat variant from human: the sinoatrial node is located in the superior caval vein above its junction with the right atrium, rather than in the atrium itself. The configuration of the mammalian AV node–His system from the AV region to the bundle branches appears to be conserved from small to large mammals. The size of the AV-node increases with the size of the heart albeit not proportionally [9]. Purkinje cells are not apparent by histology, with the ramifications of the bundle branches merging directly with the myocardium [10].

Myocyte fiber orientation has also been studied in the mouse heart [11]. In the heart of C57BL/6J mice myofibers in the middle layer appear to run mainly circumferentially, whereas those in the inner and outer layers run parallel or oblique to the apical-basal axis. Myocyte disarray is not present in the adult heart of C57BL/6J mice. Myofiber disarray, which is the histological hallmark of familial hypertrophic cardiomyopathy, was however described, in transgenic mouse models. Transgenic lines harbouring human mutations in the myosin heavy chain gene [12,13], and transgenic mice overexpressing a constitutively active Ras gene driven by the ventricular myosin light chain 2 promoter, exhibit cardiac hypertrophy and dilatation in addition to fiber disarray [14].

Coronary artery anatomy in the mouse is comparable to that of other mammals, with early branching of a large septal coronary artery (also seen in hamsters and rabbits) from the left coronary system. The diameter of the mouse coronary arteries at their ostia averages 0.16 mm [15]. By contrast, the diameter of epicardial coronary arteries (right, anterior descending, circumflex) in young adult humans averages 3.70 mm [16]. Controversy exists on the origin and importance of the circumflex branch in the mouse. The existence of a minor or rudimentary circumflex coronary artery was suggested by Michael et al. [17] in male FVB mice. In 6 Swiss adult mice, we visualised the coronary system through a casting technique, and found a single left coronary artery, which branches into a large septal artery and left anterior descending artery, supplying the left ventricular free wall. In addition the right coronary artery branches proximal into a right ventricular and circumflex vessel supplying the left ventricular posterior wall. The presence of a large septal branch explains the development of a restricted anteroapical infarction after ligation of the left anterior descending artery of the mouse heart, as illustrated in Fig. 1 [18].

2.2. Cardiac dimensions

The heart weight of an adult mouse shows considerable strain differences, and varies between 150 mg in FVB mice [19] to 180 mg in Swiss mice. This implies a heart to body weight ratio of approximately 5.0–6.0 mg/g. The mouse body weight is approximately 0.05% of the average adult human. The heart weight is proportionally reduced in size, although 20% smaller than predicted from the regression equation developed by Stahl [14,20]. In transgenic and gene targeted mouse lines the heart weight and body weight can either increase or decrease. For instance the heart weight of angiotensin II type 2 receptor deficient mice is reduced to approximately 100 mg, resulting in a heart to body weight ratio of 3.75 mg/g [21]. A significant
increase in heart to body weight ratio to 7 mg/g was found in transgenic mice overexpressing a constitutively active \( \alpha_{1a} \)-adrenergic receptor in the heart [22].

Ventricular dimensions are only sparsely described in literature. In hearts from adult Swiss mice fixed in systole we found a left ventricular diameter of 2.0 mm, left ventricular wall thickness of 1.5 mm, and an intraventricular septum thickness of 1.3 mm (Fig. 1). For C57BL/6J mice these data are 2.0 mm, 1.1 mm and 1.0 mm, respectively. The hearts were studied after perfusion fixation at a pressure of 100 mmHg. The cardiac dimensions as heart weight and wall thickness point to important differences between various mouse strains. Total blood volume for the mouse is 2.3 ml, or 80 ml/kg (vs 60–70 ml/kg for humans) [23].

2.3. Cellular content

The volume of a single myocyte is 1.5 \( \mu m^3 \). The mouse heart contains approximately 7–10 \( \times \)10^8 myocytes. Therefore approximately 90% of the left ventricular tissue volume in adult FVB mice is occupied by cardiomyocytes [19]. Although the myocytes make up the major part of the ventricular tissue volume, they are outnumbered by the interstitial cells since, in adult mice 85% of the ventricular cells are interstitial cells [24]. In a neonatal mouse only 13% of all cardiac cells are interstitial cells, indicating the plasticity of the composition of the normal mouse heart. The atria of FVB mice weigh 3.3 mg and 69% of the atrial cell number is contained by myocytes, which have a cell size of 0.225 \( \mu m^2 \). In addition, the atria contain 12% fibroblasts and 19% endothelial cells [20]. The introduction of transgenes may dramatically alter the numbers. For instance cardiac overexpression of the AT1 receptor resulted in considerable atrial hypertrophy and hyperplasia, without myocyte hypertrophy, nor changes in relative cell density [20].

The contractile function of atrial tissue strips is superior to ventricular tissue. This functional difference correlates to the ratio of phosphohamann relative to sarcoplasmatic reticulum Ca^{2+} ATPase (SERCA) II expression. Relative to SERCA II protein less phosphohamann protein was detected in the atria (0.23) than in the ventricles (0.97) [25].

The capillary/fiber (C/F) ratio determines efficiency of perfusion in cardiac tissue. C/F ratios of the heart differ between mammalian species. In the dog C/F ratios of 1.05 have been described [26,27]. In the rat heart the C/F ratio is 1.11 [28]. Our own preliminary data in Swiss mice indicate a ventricular C/F ratio of 1.45, suggesting that the C/F ratio is inversely related to the size of the animal. The higher C/F ratio could be crucial for the higher energy requirement in small animals. In adult Swiss mice we found an interstitial collagen content of 1.7%. In C57BL/6J mice even lower amounts were found, varying from 0.01% to 0.5% [14].

2.4. Cardiomyocyte metabolism

Hearts of smaller mammals have higher energetic requirements (per unit time and body mass) than larger animals, with a commensurate increase in oxidative capacity, myosin ATPase activity, and SERCA activity [29]. Part of the increased energy requirement may be met by the increased volume density of mitochondria in the myocardium (37.9% for mice vs. 25.3% for humans), with a comparable volume density of myofibrils (52.5% vs. 52.3%, respectively) [30]. Another part of the increased energetic requirement is met by higher maximal activity of enzymes involved in substrate metabolism in smaller mammals [29].

Cardiac myosin heavy chain isoforms are expressed in different patterns in rodents and humans. Although both express alpha and beta isoforms, as homodimers (\( \alpha \alpha \) or \( \beta \beta \)) or heterodimers (\( \alpha \beta \)), the predominant ventricular isoform in mice is \( \alpha \alpha \), resulting in a higher myosin ATPase activity compared to humans, in whom the \( \beta \beta \) dimer is predominant [31].

2.5. DNA synthesis in cardiomyocytes

DNA synthesis is high in the embryonic mouse heart (C3Heb/FeJ mouse) and peaks at embryonic day 12, when 33% of myocytes label after a single injection of \( ^{3} \)H-thymidine [32]. Myocyte DNA synthesis drops to approximately 1% around birth. A second, albeit lower, peak of 7.5% is found around day 7 after birth. In adult mice, DNA synthesis in the cardiomyocytes is estimated as low as 1 per 25,000 myocytes per day [24]. In the neonatal period most cardiomyocytes are mononucleated. The frequency of binucleated cardiomyocytes rapidly increases after birth, reaching values above 75% in 3 week old animals [24]. Also here the genetic background of different strains influences the number of binucleated cardiomyocytes, which varies from 3% in adult SJL/J mice to 13% in SWR/J mice [24]. Cardiac overexpression of a dominant positive Cyclin D1 protein resulted in sustained DNA synthesis in cardiomyocytes and in abnormal patterns of multinucleation [33]. Interestingly in C3Heb/FeJ mice cardiomyocyte DNA synthesis and nuclear number seem not to be affected by (isoproterenol induced) cardiac hypertrophy [32].

We measured cumulative BrdU uptake, which provides an alternative indicator of DNA synthesis, in the adult mouse.
mouse. Cumulative BrdU uptake was higher in non-myocytes (endothelial cells and fibroblasts) than in myocytes. When measured cumulatively over one week, BrdU labelling frequencies in the ventricular septum vary from 4% to 1.5% in C57BL/6J mice and Swiss mice respectively.

There are few quantitative data on the degree of apoptosis in the normal mouse heart, but apoptosis has been described in myocytes of spontaneously hypertensive mice [34] and in Coxsackievirus-induced myocarditis, where it also exhibited large interstrain differences [35]. We found no apoptosis in adult C57BL/6J mice indicating no or at least low levels of apoptosis in the normal heart. Significant numbers of apoptotic cells were observed in the first 2 weeks after myocardial infarction, especially in the border-zone between infarcted and non-infarcted myocardium (Fig. 1) [36]. These findings are in line with recent reports from studies in humans after myocardial infarction [37,38].

The cell surface receptor Fas and its ligand Fas-L, which are mediators of apoptosis, have been detected in the mouse heart [39], although Fas-L mRNA could not be detected in the heart of embryonic or adult mice [40]. These findings suggest that Fas-L protein is not synthesised in the heart under physiologic conditions.

3. Function

3.1. Mouse electrocardiography and electrophysiology

A limited number of studies have reported electrocardiograms (ECG) in mice. Initially a six lead, XYZ system was used to record a surface electrocardiogram [41]. Strain differences were reported by Goldbarg et al [41]. In adult C57BL/10 mice in vivo, the PR interval measured 44, QRS complex 12 and QT interval 88, while the RR interval was 177 ms. In isolated hearts of Swiss mice (ex vivo) we recorded: PR 38, QRS 14, and QT 64 ms at RR intervals of 154 ms (Fig. 2). The mouse electrocardiogram demonstrates significantly shorter intervals than the human hearts (PR 140-220, QRS 70 100, QT 400, RR 857 ms), which is consistent with the smaller size and higher contraction rate of mouse. No clear ST segment can be distinguished as the T wave merges with the final part of the QRS complex illustrated in Fig. 2 [41,42].

Mouse electrocardiography was taken to the next level by Berul et al. [42] by adapting the ECG recording to a 6-lead system (4 extremity leads and 2 precordial leads) and later to a 12 lead ECG [43], similar to the recording system applied in man. In addition, mouse electrophysiology (EP) was performed, by atrial and ventricular recording and pacing, in an open chest model with the leads attached directly to the epicardial surface. Furthermore, the effect of pharmacological interventions was studied by treating mice with procainamide and quinidine intravenously. Mice treated with procainamide developed second degree or complete AV block [42].

Differences in ventricular monophasic action potential recordings between mammals are predominantly due to differences in heart size, body mass, oxygen consumption, and heart rate. From shrews to humans, action potential area (encompassing amplitude and duration) is directly correlated with body mass and inversely correlated with

![Fig. 2. Bipolar epicardial electrocardiogram obtained from an isolated ejecting Swiss mouse heart. The heart rate was 390 beats/min. PR 38 msec., QRS 14 msec and QT 64 msec. Note the merging of the QT interval with the QRS complex.](image-url)
heart rate and oxygen consumption. A similar correlation is present for resting membrane potential, although the maximal upstroke velocity of phase zero depolarisation does not correlate with these parameters [44,45].

### 3.2. Phenotyping by electrocardiography and electrophysiology

Electrocardiograms were recorded in transgenic mice overexpressing SV40-T antigen under control of the ANF promoter [46], or AT$_{1A}$ receptors driven by the αMHC promoter [20]. Both phenotypes are characterised by a marked increase in atrial size. In the ANF/SV40-T antigen mice supraventricular arrhythmias were recorded as atrial fibrillation and atrial tachycardia [46]. In contrast, in the AT$_{1A}$-receptor mice slowing of conduction was recorded with a decreased firing rate of the sinoatrial node (heart rate 218 vs 360 beats/min), and prolonged atrioventricular conduction represented by a prolonged PR interval (115 ms vs 60 ms) [20]. These studies indicate the potential of the genetically altered mouse model to analyse both brady- and tachy-arrhythmias.

The adapted ECG and EP techniques were applied in a transgenic mouse model of familial hypertrophic cardiomyopathy (for review [47]). Transgenic mice were generated using the mutation ARG403GLN from the human β myosin heavy chain gene in the mouse α myosin isoform [13]. Because transgenic mice showed a high incidence of sudden death, studies were performed in heterozygous myosin heavy chain 403/+ transgenic mice to evaluate the inducibility of ventricular arrhythmias. Berul et al [43] found ventricular premature beats and inducible monomorphic ventricular tachycardia following isoproterenol infusion. They furthermore showed marked differences in QT duration in wild type 129/BS mice compared with transgenic animals. In wild type mice the refractory period was comparable in left and right ventricle. There was an increased dispersion of the refractory period comparing right and left ventricle (62.5/67.5 ms) in transgenic mice. This increased dispersion of the refractory period is considered to be a prerequisite for polymorphic ventricular tachycardias. Polymorphic ventricular tachycardia and ventricular fibrillation are the cause of sudden death in human. Polymorphic arrhythmias were however not documented in the transgenic mice. Whether the variability in refractory period contributes to the development of ventricular fibrillation as the cause of sudden death remains to be established in mouse [43].

Mouse models have been generated harbouring various ion channel mutations [48] to study the electrophysiologically mechanisms of torsade de pointes, an arrhythmia causing sudden death in patients suffering from the long QT syndrome. EP phenotyping of these mice requires measurements of QT interval and QT dispersion on the surface ECG, in concert with determination of the refractory period in different compartments during EP. Recently, Jeron et al. generated a transgenic FVB mouse line with a mutated mouse potassium channel gene (Kv1.1). The mutation in the Kv1.1 gene introduced a premature stop codon, and resulted in a truncated potassium channel protein. The truncated protein appeared to behave as a dominant negative factor in functional potassium channel formation in vitro [49]. In anaesthesised transgenic mice, the ECG was recorded and a significant increase in QT interval duration was measured in transgenic mice compared to wild type (116 versus 85 ms) [50]. Apparently the presence of the truncated protein in addition to the normal mouse potassium channel is sufficient to result in an altered phenotype, as recognised by ECG. However, despite the application of continuous heart rate monitoring no sudden death was reported.

An alternative approach was followed by Chevalier et al. 1A. They generated a minimal potassium (minK) channel deficient mouse-line. Mutations in the human minK gene can result in prolongation of QT duration and the long QT syndrome. The minK protein interacts with the LQT1 potassium channel protein and is responsible for the slow onset delayed rectifying potassium current (I$_{Ks}$), contributing to repolarisation [52]. Surprisingly, they recorded a reduction in QT duration (61/54 ms) and a reduction of the RR interval. Thus, the minK deficient mice do not provide the expected model for the human syndrome recognised by QT prolongation. Treatment with class III antiarrhythmic drug amiodarone does lead to increased QT duration in normal but not in minK deficient mice. Further evaluation of this model may contribute to the understanding of the role of the minK protein in potassium channel formation and function [51].

### 3.3. Cardiac function ex vivo

Classic ex vivo cardiac physiology preparations such as the isolated ejecting heart [7] and mechanical studies of contractility performed on isolated cardiac papillary muscles [53] are feasible in the mouse. Hearts from larger mammals display a positive force-frequency effect; that is, with increasing stimulation frequency, the force generated by isolated cardiac muscle increases in these species. In rat and mouse, however, a negative force-frequency effect has been observed. Treatment of rat and mouse cardiac muscle with ryanodine, which decreases sarcoplasmatic reticulum (SR) calcium release, was used to ‘unmask’ a positive force-frequency component which is overshadowed under physiologic conditions [54]. This suggests a differential regulation of SR calcium homeostasis in these species compared with larger mammalian species.

The isolated ejecting heart method allows measurements of systolic and diastolic function such as time to peak pressure, the time to half-relaxation and the first derivatives of intraventricular pressure (dP/dt), while pre- and after-load can be controlled. Measurements in different mouse strains were performed by Grupp et al [7]. They
Table 1
Comparison of physiologic measurements in adult mice from different strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>C3H</th>
<th>FVB</th>
<th>C57</th>
<th>SW</th>
<th>129sv</th>
<th>CD-1</th>
<th>Balb/c</th>
<th>NMRI</th>
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<td>29</td>
<td>21</td>
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<td><strong>Kidney function</strong></td>
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Anaesthetics and references:
- Pentobarbital [7]
- Methoxyflurane [85,86]
- Tri-bromoethanol [23,67]
- Ketamine [71]
- Ketamine; Xylazine [71]
- Ketamine; Xylazine; Pentobarbital [67,68]

Note: SW, Swiss Webster; CO, cardiac output; SIP, systolic intraventricular pressure; EDP, End diastolic pressure; TPP, time to peak pressure per unit pressure. ±dp/dt, positive and negative first derivative of intraventricular pressure; Ket, Ketamine; T.barb., thiobutabarbital; (') refers to anaesthetic listed in column anaesthesia; (*), measurements in open chest animals.
found a spontaneous heart rate of approximately 350 beats/min. Mean aortic pressure required to maintain function was 52 mmHg and cardiac output was ±5.1 ml/min. Intraventricular pressures were recorded with a fluid filled catheter and values ranged from 70 to 98 mmHg during systole, −2 to −9 in diastole and +3 to +9 as end diastolic pressure. The values of the first derivative of intraventricular pressure and the variation between different mouse strains are listed in Table 1. Stroke volume was 15 μl and cardiac work 270 mmHg/ml/min.

Close interaction between SERCA II and phospholamban determines the calcium uptake by the sarcoplasmatic reticulum. Several reports indicate significant changes in SERCA II and phospholamban expression levels and protein function in animal models [6] and patients [55,56] with cardiac failure. In gene targeting studies, in which one allele of the phospholamban gene was removed, the cardiac function was measured in ex vivo ejecting hearts [7]. Heterozygous phospholamban knock-out mice showed enhanced contractility [57]. This effect was accentuated in homozygous knock-out mice in which a further increase in contractile function was documented [58]. In addition, the response to β-adrenergic stimulation (isoproterenol) was reduced. These studies indicate that phospholamban regulates contractile function in the mouse heart and mediates the influence of adrenergic stimuli on cardiac contractility.

The isolated ejecting heart was also used to evaluate the protective effect of extracellular superoxide dismutase (EC-SOD) in transgenic mice (C6B3F1) following ischaemia-reperfusion. The β-actin promoter was used to drive EC-SOD expression resulting in a 3.5x increased SOD protein expression level. After 7 min of ischemia higher cardiac output and stroke volume were measured in transgenic hearts. Furthermore, improved dP/dr and stroke work were calculated in transgenic compared to normal hearts [59].

Moreover, techniques for isolation and culture of adult mouse ventricular cells permit in vitro studies on the cardiac myocytes of genetically manipulated mice, including analysis of single cell contractility and calcium fluxes, by adapting methods developed for rabbit and rat myocytes [60,61]. For instance, cardiomyocytes of phospholamban overexpressing transgenic mice were studied and found to exhibit diminished contractility and impaired relaxation, in addition to alterations in calcium fluxes (reduced amplitude and prolonged recovery phase) [61].

3.4. Cardiac function in vivo

Further characterisation of mouse hemodynamics has been achieved by measuring cardiac output and its distribution [23,62,63]. Using intraventricularly injected radioactive microspheres and the reference sample technique [64], cardiac outputs in the range of 500–700 ml/min/kg or 13–15 ml/min in conscious and tri-bromoethanol anaesthetised C3H mice have been reported. Similar values were obtained with the $^{86}$Rb-dilution method [65]. Barbee et al [23] compared cardiac output in restrained animals, during and 4 h after tri-bromoethanol anaesthesia and did not observe appreciable changes. At an anaesthetised heart rate of 425 beats/min, the stroke volume on average is approximately 30 μl, but varies between 25 and 40 μl in mice of 25–34 grams. On a body weight basis, stroke volume is around 1 μl/gram, which is similar to values observed in rats (250 μl in 250 grams animals) and humans (60 ml in 70 kg) [23]. Given the high heart rates, this implies that mouse cardiac output on a weight basis is two and nine times higher compared to rats and humans, respectively. The cardiac index of mice compared to man is 500 versus 55 ml/min/kg body weight. However, when normalising for body surface, [66] Barbee et al [23] calculated similar values in mice, rats and humans. In Swiss mice, we used electromagnetic flow probes, implanted on the ascending aorta at least one week before recording. Measurements were performed in unstrained animals (representative tracing is shown in Fig. 3) [67]. In these animals (body weights 38 g), we observed a mean cardiac output of 15 ml/min; heart rate and stroke volume were 609 beats/min and 25 μl respectively. The difference with the values obtained with microspheres in other studies can be explained by mouse strain dependent variation, or lack of stress in the absence of physical restraint. The values obtained by in vivo hemodynamic measurements are consistently higher than the values obtained in ex vivo experiments. Probably this difference is predominantly determined by neurohumoral mechanisms that are active in the in vivo situation.

Distribution of cardiac output is somewhat different between mice, rats and humans. Flow to the brain is only 3% of cardiac output, which is similar to rats, but 4-fold lower than what is found in humans [23]. Renal and splanchnic blood flow are 1.5–2 fold lower in mice, as
compared to rats and humans. These differences may reflect the relative weights of these tissues in the species. Mathematically, other tissues should be relatively over-perfused. However which tissues is unclear in the literature.

Blood pressure and cardiac output were measured in transgenic mice harbouring an ANF transgene. The offspring of transgenic crossed showed lower blood pressure in C3Heb/FeJ mice, as compared to C3H/HeJ mice [62]; this blood pressure reduction depended solely on a reduction of total peripheral resistance, possibly related to a reduction in haematocrit. The transgene did not affect cardiac output (15 ml/min) and blood flow distribution. Up to now this is the only report implementing in vivo cardiac output measurement in genetically manipulated mice.

3.5. Left ventricular pressure indices

Left ventricular pressure and its derivatives may provide information regarding the contractile status of the heart. Two groups have so far reported data on such measurements. The use of high-fidelity tip micromanometers (Millar Instruments, 2 F) and non-mechanical, computerised recording techniques (sampling rates >1 kHz) is imperative, as was recently demonstrated by Lorenz and Robbins [68]. Fluid filled catheters could not register the pressure waveforms reliably, and invariably resulted in damping of the signal. Similar tip micromanometers were used by others [69,70]. In the latter studies, an open-chest approach was used and catheters were inserted through the left atria in ketamine/xylazine anaesthesetised mice. This resulted in maximal dP/dt values around 4000 mmHg/s [69–71]. Lorenz and Robbins [68] used a closed-chest approach, inserting 2F catheters through the right carotid artery. In their study, normal (euthyroid) ketamine/thiobutabarbital anaesthetized FVB mice had a maximal dP/dt of 7830 mmHg/s. They argue that the closed-chest approach is superior to the open-chest measurements. We recently used a similar closed-chest approach with a smaller (1.4 F Millar Instruments) micromanometer in pentobarbital anaesthetised Swiss mice, and observed dP/dt values around 6000 mmHg/s. In addition left ventricular end-diastolic pressure was ±3 mmHg, versus ±8 mmHg reported by Lorenz and Robbins [68]. Although these differences between the latter study versus ours and previous studies in open-chest mice [69–71] may depend upon strain differences, different anaesthesia, and/or open versus closed chest, the differences could also depend upon mitral regurgitation in the experiments by Lorenz and Robbins as they used a relatively large manometer (0.67 mm diameter versus 1.2 mm aorta diameter).

The group of Lefkowitz [70] compared values for dP/dt in normal mice and transgenic lines overexpressing either β2-adrenoceptors, β-adrenergic receptor kinase (βARK) or an inhibitor thereof [69]. Although βARK-overexpression did not affect baseline dP/dt, the response to isoproterenol was depressed. In contrast, both overexpression of βARK-inhibitor or β2-adrenoceptors increased baseline as well as stimulated dP/dt. Similarly, IGF-1 deficiency through knockout of the IGF-1 gene resulted in hypertension and a 50% increase of positive dP/dt [71].

3.6. Echocardiography in mice

Echocardiography has been used to monitor cardiac dimensions and mechanics in vivo [72]. The best spatial resolution may be obtained with 2-dimensional echocardiography however, its temporal resolution is too low for the rapidly beating mouse hearts. Although this problem can be overcome by M-mode echo, the drawback is a limited spatial resolution. As reviewed by Hoit and Walsh [72], inter- and intra-observer errors for such parameters as left ventricular end diastolic (LVEDD) and systolic diameters (LVESD), fractional shortening (FS), velocity of circumferential shortening (Vcf), anterior wall thickness (AWTh), and posterior wall thickness (PWTh) are such, that they can be measured with acceptable accuracy. The same holds for aortic flow velocity, early (E) and late (A) mitral filling velocity (E/A ratio) as measured with Doppler. The E/A ratio is an index of diastolic function, and is ±4 in adult B6D2F1 mice. Significant changes in E/A ratio were documented in hyperthyroid (47% increase) and senescent mice (59% decrease) [73].

Using M-mode echocardiography, observations have been made in C57BL/6J [14,74,75] and FVB mice [76]. Data from these studies show very comparable LVEDD (±4 mm), LVESD (±2.3 mm) and aortic flow velocity of (±70 cm/s). FS is ±41%. However, FVB mice seem to have thicker cardiac walls (1.2 mm in FVB/N versus 0.7–0.8 mm in C57BL/6J). This implies an increased wall stress in C57BL/6J-mice, since blood pressures are comparable. By combining data from M-mode echocardiography and intra-aortic pressure measurement [76] systolic wall stress in FVB was calculated at ±49 g/cm². From the data on pressure and cardiac dimensions presented by others [14], wall stress in C57BL/6J can be calculated as 91 g/cm². This compares to 160 g/cm² in human [77].

β2-Adrenoceptor overexpression did result in increased heart rate, but did not affect mechanical or geometric parameters [74]. Cardiomyocyte-specific overexpression of phospholamban resulted in a decreased FS (79%) and a reduction in the normalised mean Vcf (67%) [61]. These findings were validated in phospholamban knockout mice, showing increased Vcf and aortic flow velocity, without affecting cardiac geometry [75]. These results illustrate the feasibility of echocardiography for hemodynamic phenotyping in genetically modified mice.

Two studies have made Doppler ultrasound observations in mouse embryos. Using a closed-abdominal approach with a 20 Mhz-probe, Gui et al. [78] were able to measure
aortic flow velocities in mouse embryos from days 10–19. They did not measure cardiac dimensions. With an open-abdominal approach, Keller et al. [79] monitored both dimensions and flow in 10.5–14.5 day embryos, combining video-techniques for measurement of the dimensions and Doppler measurements (20 Mhz) for flow rates. Embryonic C57BL/6J mice have E/A ratios of 0.4 and 0.43 at 15 and 19 days post coitum respectively [78]. The method of analysis of video images to study in vivo function was used successfully in retinoid X receptor α knock-out embryos as early as day 10 post coitum. For this purpose contrast (fluoresceinated albumin) was directly injected into the embryonic atrium, while the maternal blood supply was intact. Impaired left ventricular function was detected in the homozygous knock out embryos [2,80].

3.7. Blood pressure measurements

Arterial blood pressure and heart rate are probably the most accessible cardiovascular parameters in mice. Several methodologies have been developed and employed, comprising methods for indirect (tail-cuff) [81] as well as direct (intra-arterial) blood pressure and heart rate recording. Krege et al [82] found a correlation between systolic blood pressures measured by tail-cuff and intra-aortic mean blood pressures by fluid filled catheters in the same mice. The systolic pressure measured by tail-cuff, however was only 1–9 mmHg higher than the mean arterial pressure. Therefore there is a tendency to underestimation of systolic blood pressure, when using the tail-cuff method. Although tail cuff blood pressure has the advantage of being noninvasive, it is not uniformly reliable, since mice are quite active, and careful attention must be paid to body and tail temperature, as well as acclimatisation; we have seen substantial intra- and inter-individual variability with this method. With respect to intra-arterial measurements, approaches through the left carotid artery as well as the femoral artery have been used [23,83]. Although this has not been investigated in mice, the use of carotid artery catheters in rats has been known for a long time to be associated with serious complications, most notably the development of renal infaracts from micro-embolisms [84]. Thus, the carotid artery approach may only work for short-term measurements, immediately after implantation.

The unanaesthetised heart rate of the mouse varies between 480 and 540 beats/min at rest, compared with 60–100 beats per minute in humans. In Balb/c mice the heart rate increases to 600–650 beats/min during light activity and to 750–800 beats/min during stressful events like hand restraint [85]. Comparison of values for blood pressure and heart rate between different studies is hampered by at least two confounding factors, i.e. the use (and type) of anaesthetic during measurement as well as differences between strains. These are illustrated in a recent study [86] that reported blood pressure and heart rate in 6 different strains, as well as the effect of (recovery from) methoxyflurane anaesthesia and different degrees of physical activities (Table 1). Twenty-four hours after implantation of catheters, up to 26% difference was noted between blood pressures in C3H (92 mmHg) and 129sv mice (116 mmHg). Similarly, heart rates varied by 13% between C3H (476 beats/min) and CD-1 mice (539 beats/min). Anaesthesia resulted in decreased blood pressure and increased heart rate, which persisted for at least 4 hours. And although graded exercise increased heart rate without affecting blood pressure, comparison of sleeping blood pressure and heart rate to values observed during grooming did reveal drastic differences. Altogether, these data demonstrate that comparison of absolute values from different studies has important limitations, but that blood pressures and heart rates of ±100 mmHg and ±500 beats/min are to be considered physiological. Clearly these data demonstrate that the use of anaesthetics leads to inappropriate levels for blood pressure and heart rate, and that even the extremely short-acting methoxyflurane has cardiovascular effects that persist for at least 4 hours after termination of anaesthesia. Several other studies employing either single anaesthetics like tri-bromoethanol [23], urethane [87] or cocktails like ketamine/xyazine [71], ketamine/xyazine/morphine [88], and thio-butabarbital/ketamine [89] invariably report low to very low blood pressures. Blood pressure may be down to 51 mmHg during urethane anaesthesia in LACA mice [87] and a cocktail in FVB mice [88]. Low heart rates (down to 166 beats/min) in FVB mice during ketamine/xyazine/morphine anaesthesia have been reported [88]. Apparently, short recovery periods from injection anaesthesia prohibit reliable blood pressure and heart rate measurements. This is supported by the data from a recent study where we measured blood pressure and heart rate continuously during 2 weeks through implanted catheters in Swiss mice, and did not observe physiologic diurnal variations until 4 days after surgery (representative tracing is shown in Fig. 4) [90].

The renin-angiotensin system (Fig. 5) regulates blood pressure and salt retention under physiologic circumstances. Genetic polymorphism of the human angiotensinogen gene is associated with inherited predisposition to essential hypertension [91]. Furthermore, a deletion polymorphism in the angiotensin converting enzyme (ACE) gene has been reported to be a significant risk factor for left ventricular hypertrophy, myocardial infarction and thrombotic complications following coronary angioplasty in man [92–94]. Effects of genetic interventions at many sites in the renin–angiotensin system on blood pressure and heart rate have been documented in mice. Transgenic mouse lines expressing both rat renin and angiotensinogen transgenes display significant elevations in systolic blood pressure [95]. Lines expressing either rat renin or angiotensin, did not reveal a significant difference in blood pressure [95]. Knock out of the Ren2-gene did not affect blood pressure and heart rate in 129svv mice [96], which may
reduced blood pressure and virtual abolition of the pressor response to angiotensin II [103]; in contrast, AT<sub>1B</sub>-receptor knockout did not affect blood pressure [104]. Taken together, genetic analysis of rodent and human populations support a major role for the renin–angiotensin system in the hypertensive phenotype.

### 3.8. Treadmill exercise

Recently, a modified treadmill test was introduced by Fewell et al [105] primarily to determine the cardiac phenotype of transgenic mice. The exercise test was adapted to determine the importance of various genetic interventions in the composition of cardiac sarcomeric structures. For instance the exercise capacity of transgenic mice overexpressing the ventricular myosin light chain 2 gene in atria and ventricles was compared to that of non transgenic littermates and revealed an impaired exercise performance in the transgenic lines. The difference was most pronounced at higher belt speed (27 m/min) [105]. Forced treadmill exercise protocols could be implemented depend upon the presence of 2 renin genes (Ren1 and Ren2) in most mice strains, including 129sv. In contrast, deletion of the angiotensinogen gene in C57BL/6J mice did result in hypotension [97]. The homozygous knock out phenotype was characterised by reduced survival and hypotension could be rescued by overexpression of human renin and angiotensinogen transgenes [98]. In accordance with these observations, in ACE deficient mice [99–101] reduced blood pressures were registered. Three different angiotensin (AT) receptors have been cloned so far, the AT<sub>1A</sub>, AT<sub>1B</sub> and AT<sub>2</sub> receptor. In separate experiments all three receptors were disrupted by gene targeting. The resulting phenotypes were distinct. Two different groups generated an AT<sub>2</sub>-receptor deficient mouse and reported conflicting results on resting blood pressure, varying from no change [21] to a significant increase [102], although both studies did show an exaggerated pressor response to angiotensin II.

AT<sub>1A</sub>− [103] as well as AT<sub>1B</sub>-receptor [104] knock out lines have been generated. AT<sub>1A</sub>-knockout results in

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**Fig. 4.** Trend recording of mean arterial blood pressure and heart rate from one chronically instrumented wild type Swiss mouse. No diurnal variation was observed the first 4 days after surgery performed under pentobarbital anaesthesia. Thereafter diurnal patterns in the parameters become evident.

3.9. Kidney function

There are small differences in electrolyte serum levels comparing mouse with man. For NMRI mice values of 148 mmol/l and 4.8 mmol/l were reported for sodium and potassium respectively [89]. In man the upper limits of normal are 5.0 mmol/l for potassium and 142 mmol/l for sodium. Kidneys of C57BL/6J mice weigh 234 mg, which is 0.87% of the body weight (27 g). The mean arterial pressure in these mice was 73 mmHg [108]. Human kidneys weigh approximately 150 g, which is ±0.2% of body weight. The glomerular filtration rate (GFR) has been determined in thio-butabarbital anaesthetised mice by using 14C labeled inulin. The inulin was injected through the jugular vein, while the arterial blood pressure was registered. At regular intervals urine production was assessed. The GFR was calculated at 267 μl/min or 10 ml/min/kg body weight and 1.14 ml/min/g kidney weight. In human these values are 125 ml/min, 1.8 ml/min/kg and 0.83 ml/min/g respectively [108]. These figures suggest a comparable volume handling per gram kidney tissue in mouse and man.

Fatal kidney disease has been reported in transgenic mice overexpressing human endothelin-1 (ET-1) driven by its natural promoter [89]. Transgenic ET-1 mice develop renal cysts, interstitial fibrosis and glomerulosclerosis between 3 and 14 months of age. Interestingly, overexpression of the human ET-1, albeit modest, did not induce
hypertension despite the presence of renal pathology. The ET-1 deficient phenotype was similarly surprising, as the heterozygotes revealed an increased blood pressure instead of the anticipated reduction [109]. Creatinine blood levels or blood urea nitrogen levels were used by several investigators to judge renal function [99,110]. Esther et al [99] generated homozygous angiotensin converting enzyme (ACE) deficient mice using one construct in homologous recombination to destroy both somatic and testicular ACE. In wild type C57BL/6J mice a creatinine level of 0.30 mg/dl was found, compared with 0.56 mg/dl in the ACE deficient mice. In addition, a significant reduction in systolic blood pressure was recorded in these animals by the tail cuff method (73 mmHg in homozygous knock out, versus 110 mmHg in wild type) [99].

The technique to evaluate the function of the homeostatic tubuloglomerular feedback (TGF) loop was adapted to the mouse level by Schnermann et al [111]. They were able to determine the effect of changes in the loop of Henle flow rates on stop flow pressure ($P_{SF}$). This technique requires distal obstruction of one loop of Henle in addition to the introduction of pipettes in the proximal segment. In wild type mice the $P_{SF}$ drops with increasing flow rates. This physiologic response is completely blocked in homozygous AT$_{1A}$ deficient mice. Thus, the tubuloglomerular feedback requires intact AT$_{1A}$ receptors in the glomerular vasculature. The same study calculated the single nephron GFR in mice, using $^{125}$I-iothalamate. They found a late proximal flow rate of 15.8 nl/min, and late distal flow rate of 13.7 nl/min.

4. Experimentally induced pathology of the cardiovascular system

In principle, most manipulations in the cardiovascular system which previously have been performed in larger rodents and other experimental animals can be adapted to the mouse. The limitations are those of scale and operator experience; microsurgical techniques must be perfected to permit reproducible vascular manipulation and vascular anastomoses in vessels less than 0.5 mm in diameter. Bearing this in mind, mouse models of cardiac pressure and volume overload, ischaemic cardiac damage, hypertension, and others have been created.

4.1. Left ventricular hypertrophy: transverse aortic banding

Banding of the transverse aorta results in pressure overload and left ventricular hypertrophy [2,112]. This technique was initially validated by Rockman and co-workers [112] in wild type C57BL/6J mice, showing an increased heart to body weight ratio, in addition to reactivation of a foetal gene program, after the establishment of a pressure gradient of approximately 40 mmHg. The foetal gene program is characterised by re-expression of immediate early genes and ventricular ANF [113]. Treatment with either losartan or captopril attenuated the increase of the heart to body weight ratio [114]. These studies indicate the importance of the renin–angiotensin regulating system during pressure overload induced left ventricular hypertrophy. Aortic banding in gene targeted and transgenic mouse lines with alterations in the renin–angiotensin system may be used to elucidate the molecular mechanisms responsible for the hypertrophic response.

4.2. Right ventricular hypertrophy and volume overload: pulmonary artery banding

A modest gradient over the pulmonary artery as created by pulmonary artery banding, was associated with right ventricular hypertrophy and downregulation of phospholamban both at the RNA and protein levels [6]. This downregulation was even more pronounced in right ventricular failure induced by severe pulmonary artery stenosis. ANF showed the reverse response and is upregulated in right ventricular hypertrophy and failure [6]. These findings suggest an important role for phospholamban in the regulation of contractile function in the mouse heart in vivo.

4.3. Renovascular hypertension: renal artery banding

To mimic renovascular hypertension, Wiesel et al [115] placed clips with a luminal diameter of 0.12 mm on either one or both renal arteries of wild type C57BL/6J mice. The C57BL/6J was deliberately chosen for these experiments as they only express the Ren-1 gene. Two different models were studied, the two kidney-one clip (2K1C) and the one kidney–one clip (1K1C). The 2K1C treated group showed increased MAP compared with sham operated mice (135 versus 116 mmHg) with persistently increased plasma renin levels (approximately threefold) four weeks after surgery. The 1K1C group developed even higher pressures (153 versus 116 mmHg), without a significant change in the renin levels. These results are similar to the findings reported previously in rats [116,117]. Cardiac hypertrophy was most pronounced in the 1K1C group, where the heart to body weight ratio increased from 4.0 to 5.3 mg/g. In the 2K1C group the ratio increased to 4.4 mg/g. Therefore the two classical rat models for either renin dependent (2K1C) or volume dependent (1K1C) can be reproduced in mice.

4.4. Ischemic cardiac damage: coronary artery occlusion

In the rat, ligation of the left coronary artery has been extensively used to induce myocardial infarction (MI).
This model has turned out to be extremely versatile in the study of the pathophysiology and pharmacology of myocardial infarction and the ensuing heart failure [118,119]. A similar approach in genetically modified mice may further contribute to our understanding of the processes underlying the functional and structural responses to MI.

Also in the mouse, coronary artery ligation results in MI [17,18,36]. Michael et al. occluded the left anterior descending coronary artery in FVB mice, through a trans-sternal approach. Besides permanent occlusion, they employed ischemia for 30 or 60 min, followed by reperfusion [17]. Their procedure has a death rate of 23–38%, with the lowest rate (23%) occurring in the permanent occlusion group. Infarct size (IS) and area at risk (AAR) were determined by planimetry on 5 transverse slices, following appropriate staining. The IS relative to the left ventricular size was 28% (range 12.3 to 47.6%), while the AAR reached up to 69%. A significant reduction of IS relative to both left ventricular and AAR size was seen in reperfusion after 30 and 60 minutes of ischemia. The validity of this method of determining IS and AAR may be questioned, especially when comparing results from mice to other species. In mice, ligation of the left coronary artery typically results in antero-apical transmural infarctions [17]. In other species, like the rat, infarcts encompass the left ventricular free wall, not including the apical area (unpublished observations).

The mouse MI model was successfully implemented in experiments aimed at determining the role of apoptosis in ischaemic cell death. Bialik et al. [36] used an intercostal approach to ligate the left coronary artery in C57BL/6J mice. Apoptosis was shown to occur predominantly in hypoperfused myocardium from 4 to 48 h after left anterior descending artery occlusion. Apoptosis triggered by hypoperfusion was independent of p53 protein expression as identical results were found in homozygous p53 deficient mice [36]. In our hands, permanent ligation just proximal of the bifurcation of the left coronary artery results in acute death rates of 5–10%, whereas, during 5 weeks follow-up, another 5% died. MI results in progressive dilatation of the left ventricular cavity and thinning of the infarcted area (Fig. 1) [18]. Heart weight, and heart to body weight ratios are essentially unaffected, which implies hypertrophy of the remnant myocardium. In contrast to rats, this is, however, not reflected in an increased interventricular septal thickness. As in rats [120], infarction is associated with increased DNA-synthesis in interstitial cells and excessive collagen deposition in the remnant myocardium. Functional analysis of left ventricular pressure and its derivatives following MI in closed chest animals at 1–5 weeks revealed depression of systolic pressure, positive and negative dP/dt; in contrast to what has been noticed in other species, LVEDP remained normal, up to 5 weeks after MI. This suggests a buffering, windkessel-like function of the apical aneurysm. Thus, the classical circulatory congestion of heart failure is not observed in mice.

5. Future directions

This review summarises the physiologic tests available to characterise the phenotype of genetically modified mice. Through down-sizing of technology, basically every test available in humans can be performed in mice today, with a few exceptions. Human pathologic conditions can be mimicked in mice by microsurgical and pharmacological interventions. In addition terminal tests can be performed in the mouse model to study cardiac function ex vivo and to isolate cardiac cells for in vitro studies. An important limitation of the mouse model is the marked difference in physiologic parameters between various mouse strains (listed in Table 1). Therefore, care should be taken in selecting the appropriate strain for each experiment. Marked differences were reported related to anaesthesia and time delay following surgical procedures. Results obtained in the acute experiment may vary considerable from data acquired in chronically instrumented mice.

The most important development is the introduction of genetically manipulated mice which provide the tools to systematically unravel molecular pathways in physiologic and pathologic cardiovascular conditions. The gene targeted and transgenic models discussed so far are, however relatively crude genetic manipulations leading to pronounced phenotypical changes.

The challenge for the next decade will be the development of reliable inducible cell type specific promoters, to direct transgene expression to the cell type of choice at any desired moment in predestined circumstances [121,122]. The tetracyclin-dependent transgene expression is an example of such an approach. The introduction of the Cre recombinase LoxP system in gene targeting technology [123] has been proven to function in the mouse heart, and makes the generation of tissue specific knock out experiments feasible [124]. By placing the recombinase gene under control of a tissue specific tetracycline dependent promoter the moment of the gene deletion can be carefully chosen [125]. This fine tuning of the genetic changes, in concert with adaptations in physiological technology will help to design experiments with even more relevance to the human situation.

References


