Autonomic control of blood pressure in mice: basic physiology and effects of genetic modification

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Janssen, Ben J. A., and Jos F. M. Smits. Autonomic control of blood pressure in mice: basic physiology and effects of genetic modification. Am J Physiol Regulatory Integrative Comp Physiol 282: R1545–R1564, 2002; 10.1152/ajpregu.00714.2001.—Control of blood pressure and of blood flow is essential for maintenance of homeostasis. The hemodynamic state is adjusted by intrinsic, neural, and hormonal mechanisms to optimize adaptation to internal and environmental challenges. In the last decade, many studies showed that modification of the mouse genome may alter the capacity of cardiovascular control systems to respond to homeostatic challenges or even bring about a permanent pathophysiological state. This review discusses the progress that has been made in understanding of autonomic cardiovascular control mechanisms from studies in genetically modified mice. First, from a physiological perspective, we describe how basic hemodynamic function can be measured in conscious conditions in mice. Second, we focus on the integrative role of autonomic nerves in control of blood pressure in the mouse, and finally, we depict the opportunities and insights provided by genetic modification in this area.

autonomic nervous system; hemodynamic control mechanisms; adrenergic receptors

The development of techniques to genetically modify mammals has boosted research to identify the molecular mechanisms involved. The number of candidate genes identified by gene array studies is accelerating and need for screening is rapidly expanding. For technical reasons, the functional consequence of gain or loss of one or more genes is generally assessed in the mouse. Because of its small size, determination of the cardiovascular phenotype is not easy. In the last decade, however, many techniques that were developed to study hemodynamics in larger species have been miniaturized. In this process, studies were initially restricted to in vitro techniques. Now, in vivo approaches are feasible and aspects of cardiac and vascular function can be measured in conscious mice.

In this review, we will mainly focus on the integrative role of autonomic nerves in control of blood pressure in the mouse. After a detailed description of basic hemodynamic function in the mouse, we will discuss the genotypes that affect control of blood pressure by altering sensory, central, and efferent processing of autonomic nervous activity. The impact of anesthesia on cardiovascular parameters in the mouse is such that it could easily overshadow subtle changes induced by gene manipulation. Therefore, observations will be discussed that have been acquired primarily in conscious conditions in mice.

Basic hemodynamic characteristics in mice

Is the mouse just a small rat? Can we, by using mathematical scaling techniques, make predictions on hemodynamic characteristics in the mouse? Are all mice equal? Are reported differences between mouse strains of physiological relevance or do they mirror shortcomings of previous technology as we proceed to improve ways to monitor physiological processes in the mouse? These questions are of relevance, certainly as...
we intend to extrapolate the results of our studies in mice to the human situation. Presently, several hundred strains and substrains of mice are known, and many new inbred strains are being expected, accepting the nomenclature rules that a strain should be regarded as inbred when it has been mated brother × sister for 20 or more consecutive generations (11, 36). Even within a single strain, a large degree of genetic diversity may exist. For example, the genetic variance in mice of the 129 strain, which is of importance in creating knockout and other targeted mutant mice, was so large that the International Committee on Standardized Genetic Nomenclature for Mice introduced a new nomenclature to distinguish between the different parental lines and related 129 strains (187, 200).

In our view, differences between techniques and methodology have been a major source for disagreement in blood pressure levels between strains. However, we expect that improving technology will take away many inconsistencies, certainly in the cardiovascular area where hemodynamic parameters are known to depend heavily on experimental conditions. For instance, a recent report by Mattson (132) showed that blood pressures of five strains of mice (Swiss Webster, A/J, C57BL/6J, C3HeBFeJ, and SWR/J) were unexpectedly very comparable [range of mean arterial pressure (MAP): 108–114 mmHg], when measurements were performed in the conscious state using chronic catheterization techniques. For this reason, we avoided reviewing potential strain differences. Rather, we will focus on the opportunities that are available now to characterize hemodynamics in mice in the conscious state.

In global terms, cardiac and vascular morphology differences between mouse, rat, and human species are only subtle. Also, many physiological parameters are very comparable. This is illustrated in Table 1 in which functional physiological characteristics that have been recently measured in mice are compared with those predicted by allometric formulas (180, 188). It appears that many of the hemodynamic values observed in the mouse are within the range as predicted by the formulas, supporting the view that physiological mechanisms in mice are closely related to those of humans.

The table is completed with values for arterial pressure that are independent of scale. Parameters that can be measured in mice in the conscious state are discussed in the next section.

**ELECTROCARDIOGRAM**

In conscious mice, the most reliable indexes of cardiovascular function are obtained by telemetry. This technique offers the ability to record hemodynamics for relatively long periods of time in conscious, freely moving animals without the limitations of restraint or anesthesia (139, 204). Perhaps the most accessible measure that can be obtained in this way is the electrocardiogram (ECG). Depending on the manufacturer, the subcutaneous implantation of a single device may simultaneously render values for temperature and locomotor activity, making the device attractive for behavioral studies too (9, 82, 90, 91, 192, 221). Characteristic for the mouse ECG is that a clear ST segment is absent and that the T wave merges with the final part of the QRS complex. Mouse ECG changes in response to myocardial ischemia are comparable to those observed in rats, showing R wave enlargement and ST-segment elevation (219). Until recently, the view

**Table 1. Comparison of physiological parameters in humans and mice based on allometric scaling equations as well as in vivo measurements**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Human, 70 kg</th>
<th>Mouse, 0.03 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equation: Mb = body weight, kg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic metabolic rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O2 consumption, l kg⁻¹ h⁻¹</td>
<td>0.676 × Mb⁻⁰.²⁵</td>
<td>0.23</td>
</tr>
<tr>
<td>Lung capacity, ml</td>
<td>63 × Mb⁻¹.⁰²</td>
<td>4,801</td>
</tr>
<tr>
<td>Tidal volume, ml</td>
<td>6.2 × Mb⁻¹.⁰¹</td>
<td>452</td>
</tr>
<tr>
<td>Respiration frequency, min⁻¹</td>
<td>53.5 × Mb⁻¹.⁰₂⁶</td>
<td>18</td>
</tr>
<tr>
<td>Blood volume, l</td>
<td>0.055 × Mb⁻⁰.⁹⁹</td>
<td>3.7</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>5.8 × Mb⁻¹.⁰⁹</td>
<td>390</td>
</tr>
<tr>
<td>Heart rate, min⁻¹</td>
<td>241 × Mb⁻¹.²⁵</td>
<td>83</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>0.66 × Mb⁻¹.⁰⁵</td>
<td>57</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>166 × Mb⁻⁰.⁷⁹</td>
<td>4,761</td>
</tr>
<tr>
<td>Circulation time, s</td>
<td>17.4 × Mb⁻¹.²⁵</td>
<td>50.3</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>5.36 × Mb⁻¹.⁷²</td>
<td>114</td>
</tr>
<tr>
<td>RPF, ml/min</td>
<td>21.79 × Mb⁻¹.⁷⁷</td>
<td>574</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

References are given for mouse data only. For human data, a normal range is indicated. The equations are based on those listed in the book of Schmidt-Nielsen (180) and paper of Singer (188). Normal values as found in adult males are based on those given in Guyton’s and Hall’s textbook of medical physiology (62). CO, cardiac output; GFR, glomerular filtration rate; RPF, renal plasma flow; MAP, mean arterial pressure.
was held that the size of the mouse atrium was too small to induce fibrillation via reentrant circuits. However, as shown by Wakimoto et al. (211), sustained atrial tachycardia and fibrillation could be induced with endocardial pacing after cholinergic agonist administration. The genetic aspects and application of the echocardiography technique to assess anti-ischemic and antiarrhythmic interventions have been reviewed in detail by Wehrens et al. (219).

BLOOD PRESSURE

Telemetric recording of murine blood pressure is technically more challenging. So far, only a few reports have been published (17, 18, 60, 108, 129, 138, 139, 183). After the implantation of the pressure sensor, blood pressure and heart rates (HRs) are slightly elevated for 4–5 days (17). After this recovery period, day-night rhythms are reestablished with daytime blood pressures being ~20 mmHg lower than nighttime blood pressures. Average 24-h blood pressure values obtained by telemetry are usually in the range of 90 to 115 mmHg in wild-type control mice. Average 24-h HR values are usually between 550 and 650 beats/min. Comparable pressures and day-night pressure variations have been reported when tethering techniques were applied for continuous long-term blood pressure monitoring (88, 112, 131, 132). Also, with tethering techniques, blood pressure and HR rhythms needed 4–5 days to become fully expressed again (88).

With telemetric devices, blood pressures have been recorded up to 150 days after implant (139). With tethering techniques, the recording period is limited (~3 wk), but it allows the simultaneous implantation of a venous line and assessment of drug effects without disturbing the animal. In most studies using telemetric devices, mice have not received additional catheters and need to be handled each time a drug or substance needs to be administered. However, handling of the mouse disturbs hemodynamics considerably and may obscure acute drug effects. Even entering the test room for only 5 min has been shown to increase locomotor activity, HR, and temperature in mice for 30 min (13). In our experience, mice are more active than rats when kept in standard laboratory cages. To minimize arousal, animals are accustomed to human sounds by playing the radio in the animal rooms during daytime hours. Furthermore, mice are allowed to settle for 1 h after they have been connected to the measuring equipment. Despite these efforts, baseline hemodynamics are easily disturbed by movement of the mouse. Figure 1 illustrates the variability in hemodynamics one may observe when a mouse is actively moving through the cage. As can be seen in that tracing, blood pressure variability (within a 10-s period) can be quite large. Even when mice are sleeping, average blood pressure may vary up to 10 mmHg between nonrapid eye movement and rapid eye movement sleep stages (179). Measurements in resting conditions are required when the investigation aims to uncover subtle effects introduced by genetic or pharmacological intervention. Alternatively, long recording periods are required to identify small differences. In many experimental settings, continuous blood pressure measurements are either not feasible or unnecessary. In this type of study, blood pressure may be recorded from arterial catheters that are guided to the neck of the mouse and extended from the cage on the day of the measurement. Preferably, the arterial catheter should be inserted into the abdominal aorta via the femoral artery and not via a carotid artery, because the chance of microembolisms causing problems is much greater in the latter approach (32).

Estimates of systolic blood pressures in mice can be achieved by the noninvasive tail-cuff technique (92, 109). Mice are usually restrained and heated to obtain adequate pulsatile pressure signals in this peripheral organ that is very responsive to sympathetic influences. Individual readings of this method vary considerably and repeated measurements are required. For these reasons, this method is not very accurate in assessing true values of blood pressure in mice. Also, in rats, this method is not regarded as very reliable (81). The tail-cuff method may be useful when pressure levels between groups have to be compared repeatedly over long time periods.

CARDIAC OUTPUT

Cardiac output (CO) has been assessed in mice with various techniques ranging from traditional indicator-dilution techniques (7, 14, 166, 178, 208, 215, 220) up to noninvasive echocardiography (45, 210, 226) and
magnetic resonance imaging (222, 223). Whereas most of the dilution-based methods need multiple blood sampling, which is limited in the mouse, the noninvasive imaging techniques cannot be applied continuously and are in many cases performed under anesthesia. Because most anesthetic drugs reduce HR by more than 200 beats/min, reliable estimates of CO have not been achieved yet in conscious mice. Recently, we used miniaturized transit time flow probes as well as electromagnetic flow probes to measure ascending aortic flow in adult conscious mice weighing 30 to 40 g (87). In that study, stroke volume (SV) ranged from 20 to 46 μl and CO from 12 to 27 ml/min. When normalized for body weight, the average (means ± SE, n = 7) SV index and cardiac index amounted to 846 ± 173 μl/kg and 532 ± 103 ml·min⁻¹·kg⁻¹, respectively. Now, we are also able to implant these probes in mice weighing 20–30 g (Fig. 2). In mice, the cardiac index is nearly twice the value found in rats and even 10 times greater than observed in humans (see Table 1). These differences are entirely due to HR. SVs are ~1 μl/g body wt across the three species. Thus, in an adult mouse with a blood volume of 2.5–3 ml (70 ml/kg) and a CO of 20 ml/min, blood is rapidly recirculating seven to eight times per minute. These hemodynamic characteristics fit the allometric predictions (Table 1).

To characterize cardiac function, we explored ways to record maximal values of CO in mice. Exercising instrumented and tethered mice is difficult. Therefore, we chose to stimulate CO by increasing circulating volume. This is achieved by infusing intravenously warmed Ringer solution at a rate of 2.5 ml/min for 40–50 s. Alternatively, CO can be increased by infusing the inotropic agent dobutamine. Applying both stimuli in C57Bl6 mice, we observed maximal CO values being 30–45% above their resting values (Fig. 2). Remarkably, the increase of CO induced by volume loading appeared to depend on a rise in SV rather than a rise in HR, whereas during dobutamine infusion, the reverse was true. These findings suggest that the present protocols may underestimate true maximal values of CO. Evidence from studies on force-frequency modulation of heart function in the mouse (54) supports the view that ventricular filling is a critical determinant of CO. With the use of miniaturized pressure-volume catheters, it was found that end-diastolic volume declined progressively with increasing HRs in mice (54). Therefore, at HRs above 700, a limited ventricular filling may prevent a further increase of CO and cardiac contractility. This may also be the reason that, in contrast to humans in whom CO can increase four to five times, mice have a limited ability to increase CO.

Because of the limited cardiac reserve, rapid changes in venous return may influence CO and arterial blood pressure considerably. To explore this further, we examined the effects of intravenous bolus injections of fluid on hemodynamics in mice. As can be seen in Fig. 3, following a 100-μl bolus injection of saline, maximal changes in pressure and HR were relatively small. However, SV and CO increased for a few seconds up by 10–15%. In addition, we repeatedly observed that in actively moving mice, blood pressure may fall within seconds by more than 20 mmHg, especially when the animal stretches its body forward or compresses its thorax otherwise. As indicated by the arrows in Fig. 1, there is a concomitant fall in SV, CO, and MAP, which is not buffered by acute changes in HR. To quantify to which extent variations in CO depend on those occurring in HR or in SV, we computed coherence values between HR and CO on the one hand and SV and CO on the other (87). The results indicated that, at frequencies lower than 0.1 Hz, fluctuations in SV and HR contribute evenly to fluctuations in CO. At frequencies above 0.1 Hz, however, variations in CO were mainly determined by those occurring in SV. Thus, in mice, control of CO seems to be hampered by the sensitivity of SV to rapid changes in venous return (e.g., behaviorally induced) and limited ventricular filling at high HRs. However, it should be noted that this accounts

Fig. 2. CO, SV, MAP, and HR as measured in resting conditions (Rest) in conscious C57BL6 mice (n = 6) instrumented with transit time flow probes placed on the ascending aorta. To stimulate CO (Stim), measurements were also made during volume loading (intravenous Ringer solution 2 ml in 1 min, solid bars) and during intravenous infusion of dobutamine (0.5 μg·kg⁻¹·min⁻¹). Values in the open bars give the maximally induced changes under these conditions. Values are means ± SE. Values obtained during volume loading were compared with those obtained with dobutamine using unpaired t-tests. Statistical significance was accepted at *P < 0.05.
only for very short-term fluctuations and is probably not distressing the mouse because of its horizontal position. As we will illustrate further below, the response time of reflex mechanisms is such that they are unable to buffer these fast beat-to-beat changes in hemodynamics.

**CARDIAC DIMENSIONS AND EJECTION FRACTION**

Although echocardiography and Doppler techniques are usually applied in the anesthetized state, some groups are now able to use these techniques in conscious restraint conditions (76, 86, 165, 196, 227). In this condition, CO is comparable to the stimulated values observed by our direct measurements (87). Pentobarbital sodium and ketamine/xylazine depress cardiac function parameters by more than 30% (196, 227). Especially HR can be severely reduced by high doses of ketamine/xylazine. According to Hart et al. (65), murine cardiac function becomes abnormal when HR is lower than 300 beats/min. If measurements in conscious conditions are not feasible, then volatiles (196) -chloralose-urethane-based (55) anesthetic regimens are a reasonable alternative to sustain HR. Various aspects of the echocardiographic technology and its application in mouse studies have recently been reviewed by Hoit (76).

**REGIONAL BLOOD FLOWS**

Distribution of CO has been assessed in mice using microspheres (6, 7, 142, 166, 178, 215). The mouse brain receives ~2–4% of CO (166), whereas in humans, 11–13% is found. However, given the relative smaller brain size of the mouse, this is not surprising. Splanchnic blood flow and renal blood flow (RBF) were 14 and 11% of total CO, respectively. These values are lower than those generally observed in humans (25 and 20%, respectively), suggesting that other organ beds are relatively underperfused in mice. The insertion of a catheter into the left ventricle may be stressful and may be associated with renal and mesenteric vasoconstriction, certainly when these measurements are performed in conscious conditions (6). In general, indicator-dilution techniques require repeated sampling from body compartments. Given the sensitivity of CO to volume changes, the results should be interpreted with care. Recently, Hallemeesch et al. (63) refined such methods and were able to determine metabolic fluxes and blood flow across several organs without waste of blood. Values for liver, renal, and hindquarter blood flow by their methods were 1.2 ± 0.3, 1.0 ± 0.1, and 1.1 ± 0.3 ml·10 g body wt⁻¹·min⁻¹, respectively.

RBF estimates obtained by microspheres or paraaminohippurate clearance techniques vary roughly between 1 and 2 ml/min (23, 166), which is ~10–20% of CO. In terms of flow per gram kidney weight, values ranging from 3 to 8 ml·min⁻¹·g⁻¹ have been found. Comparable values have been obtained by direct measurements of RBF using miniaturized transit time flow probes placed around the main renal artery (2, 59, 61, 138). Up to now, this was only possible in anesthetized conditions. Very recently, Callahan et al. (unpublished observations) were able to implant such a probe chronically in a mouse allowing continuous RBF measurements in conscious conditions (Fig. 4). Glomerular filtration rate (renal inulin clearance, anesthetized conditions) ranges from 0.7 to 1 ml·min⁻¹·g kidney wt⁻¹ (15, 153, 154). When scaled to body weight, renal function parameters of mice and humans are comparable (188) (see Table 1). This suggests that volume handling by the kidney is similar in mice and humans and makes the mice apt to study human pathology. The renal physiology of the mouse has been reviewed in more detail by Meneton et al. (135) and Lorenz (119).

**WEBSITES ON MOUSE PHYSIOLOGY**

In the past few years, websites are emerging that are part of commercial or scientific initiatives, and they display a variety of information on mouse physiology. In Table 2, we listed some of these sites that may be of help for researchers interested in mouse physiology. The topics vary widely and on these pages useful links to other sites are often supplied.
Table 2. List of websites with additional information on scientific and methodological aspects of mouse physiology

<table>
<thead>
<tr>
<th>Web Address</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="http://www.rodentia.com/wmc/index.html">http://www.rodentia.com/wmc/index.html</a></td>
<td>This site serves as a central place to find numerous Internet resources of particular interest to scientific researchers using mice or rats in their work</td>
</tr>
<tr>
<td><a href="http://www.informatics.jax.org/nominal/other/mouse_facts3.shtml">http://www.informatics.jax.org/nominal/other/mouse_facts3.shtml</a></td>
<td>General information on mouse physiology</td>
</tr>
<tr>
<td><a href="http://www.informatics.jax.org/nominal/nomen/strains.shtml">http://www.informatics.jax.org/nominal/nomen/strains.shtml</a></td>
<td>Nomenclature of mouse strains</td>
</tr>
<tr>
<td><a href="http://www.eulep.org/Necropsy_of_the_Mouse/index.php">http://www.eulep.org/Necropsy_of_the_Mouse/index.php</a></td>
<td>Autopsy techniques, diagnostic information on mouse diseases</td>
</tr>
<tr>
<td><a href="http://www.nervenet.org">http://www.nervenet.org</a></td>
<td>Mouse brain library</td>
</tr>
<tr>
<td><a href="http://www.uio.edu/~vpr/research/animal/mice0001.htm#General_Biology">http://www.uio.edu/~vpr/research/animal/mice0001.htm#General_Biology</a></td>
<td>Images of brain sections and movies</td>
</tr>
<tr>
<td><a href="http://www.mousespecifics.com/">http://www.mousespecifics.com/</a></td>
<td>Biomethodology of the mouse</td>
</tr>
<tr>
<td><a href="http://www.mousephysio.com">http://www.mousephysio.com</a></td>
<td>Site that offers help in technologies for genomics and data analysis (i.e., ECG)</td>
</tr>
<tr>
<td><a href="http://ccm.ucdavis.edu/tvmouse/">http://ccm.ucdavis.edu/tvmouse/</a></td>
<td>Information on mouse physiology, including instrumentation techniques</td>
</tr>
<tr>
<td><a href="http://www.rodentia.com/wmc/index.html">http://www.rodentia.com/wmc/index.html</a></td>
<td>Introduction to the anatomy, physiology, histology, and pathology of the laboratory mouse with an emphasis on genetically engineered mice</td>
</tr>
</tbody>
</table>

Please note that the choice of links is subject to the author’s subjective opinion and certainly not comprehensive.

ASSESSMENT OF AUTONOMIC FUNCTION IN MICE

The autonomic nerves are crucial for maintaining cardiovascular homeostasis. However, assessment of autonomic nervous activity in mice is complicated. The approaches that have been used in this species are summarized below.

MEASUREMENT OF CATECHOLAMINE CONCENTRATIONS IN PLASMA

Traditionally, sympathetic activity has been assessed by measuring plasma or tissue catecholamine levels. Plasma norepinephrine (NE) concentrations range widely in mice. Values as low as 0.2 ng/ml (101) up to 70 ng/ml (79) have been reported. In the first study, the method of blood sampling was not explained; in the latter study, blood was taken under ether anesthesia. When blood is sampled via arterial catheters, plasma NE concentrations usually range between 0.5 and 2 ng/ml (70, 126, 127). Other groups have reported baseline plasma NE values ~5–15 ng/ml (35, 69, 234). From these data, it seems that plasma NE levels are about a factor 3 to 10 higher in mice than in rats or humans. Whether this elevation is due to artifacts in taking the blood samples or due to real species differences is not clear. The methods of blood sampling and types of anesthesia do certainly influence baseline catecholamine levels. On the basis of the present data, it cannot be concluded whether circulating catecholamines are really elevated in mice and indicative of enhanced ongoing sympathetic nerve activity.

DIRECT MEASUREMENTS OF AUTONOMIC NERVE ACTIVITY

In the mouse, direct measurements of sympathetic nerve activity have been obtained under anesthetized conditions only (117, 229). Baroreceptor and chemoreceptor control of renal sympathetic nerve activity (RSNA) were compared between rats and mice during urethane anesthesia (117). Although HR was ~200 beats/min higher in the mouse than in the rat, basal RSNA was reduced in the mouse. Obviously, the renal nerves are thinner in the mouse than in the rat. There is no information about the caliber or number of axons of renal nerves in this species. Therefore, it is difficult to decide from such multifier preparations whether the reduced firing frequency is real or merely reflects the lower number of axons in a preparation.

In relative terms, RSNA responses to baroreceptor loading and unloading were comparable in the mouse and in the rat. Only the baroreflex gain (in %ΔRSNA/mmHg) was slightly enhanced, being 2.4 ± 0.2 in mice vs. 1.9 ± 0.1 in rats. RSNA changes evoked by hypoxia and hypercapnia were quite similar in both species. It was recently shown that in mice ongoing levels of RSNA may depend on circulating levels of ANG II (122). Ma et al. (123) showed that ANG II was able to increase RSNA substantially before and after ganglionic blockade, suggesting that the hormone may directly activate postganglionic renal sympathetic neurons. Although no data were shown, they reported that similar effects could also be elicited by ANG II injections in rats. This novel effect of ANG II was recently confirmed by studying the cellular responses to ANG II in subpopulations of sympathetic neurons isolated from murine aortic-renal ganglia. Thus, at least in the mouse and rat, locally produced or high circulating levels of ANG II may influence ongoing RSNA and hence medate renin release, sodium reabsorption, and RBF. To which extent this mechanism is involved in controlling renal hemodynamics in conditions of elevated ANG II levels has not been established yet. Baseline plasma ANG II concentrations are ~20 fmol/ml in mice (28, 37, 189) and do not differ much from values obtained in humans. Thus circulating levels of ANG II do not seem to be responsible for the suggested elevated sympathetic activity in the mouse.

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ASSESSMENT OF BARORECEPTOR REFLEX FUNCTION

The baroreflex is one of the most potent regulators of blood pressure. This reflex buffers blood pressure fluctuations by adapting CO and vascular resistance and is of eminent significance for adequate perfusion of the brain, especially in species that are erect most of the time. In the mouse, baroreflex-mediated parasympathetic and sympathetic control of HR can be demonstrated by applying the classical technique of injections of short-term vasoconstrictor or vasodilator agents. Bradycardic responses to an intravenous bolus injection of phenylephrine are found invariably. However, the rise of HR in response to a bolus injection of nitroprusside is easily masked when the animal is aroused or when resting hemodynamics have been disturbed otherwise (159). As illustrated in Fig. 5, following an intravenous bolus injection of phenylephrine, HR decreased within 1 s to near minimal values after the initiation of the pressure ramp. In response to sodium nitroprusside, HR increased even before the pressure fall occurred. However, the rate of change in HR was much slower than following pressure increments. In this example, following phenylephrine injection, HR returned to near control values within 5 s before it fell again, yet, less outspoken. From the bimodal characteristic of the HR response, one may infer that the relatively fast parasympathetic (activation) was followed by a slower sympathetic (withdrawal) response. However, in the presence of atropine, HR never decreased (88). Thus, at least in our setup, HR responses to rapid pressure increments are mainly parasympathetically mediated and not due to sympathetic withdrawal. The bimodal temporal pattern is probably the result of a pharmacokinetic phenomenon. Given the fast circulation time of the blood in a mouse (~5 s), it is likely that after the first passage of phenylephrine, the agent reappeared in responsive tissue before its distribution was complete and, hence, caused a secondary increase in blood pressure and consequent decrease in HR.

Baroreflexes have also been studied in mice using short-term (~2 min) infusions of vasoconstrictor and vasodilator agents to produce gradual ramps in blood pressure (136). The advantage of this method above bolus injections is that sufficient time is allowed for full sympathetic adjustments. In addition, this method allows the construction of sigmoidal curves to assess limits and gain of the cardiac baroreflex (117, 136). Unlike for the rat (68) or rabbit (167), the effect of blockade of parasympathetic and sympathetic components has not been studied in mice. There are no data yet demonstrating the feasibility of chronic arterial baroreceptor denervation in the mouse. Only in acute preparations, by whole vagotomy, baroreceptor-independent renal sympathetic reflexes have been studied (122).

ASSESSMENT OF CARDIOVASCULAR RESPONSES TO AUTONOMIC BLOCKING AGENTS

The contribution of autonomic nerves to blood pressure control has been examined by recording hemodynamic changes to autonomic blockers. In conscious mice, acute ganglionic and β-adrenoceptor (AR) blockade decreases baseline blood pressure by ~20 mmHg (88, 95). Blood pressure is not altered by acute muscarinic receptor or β-AR blockade in mice. These blood pressure responses are not different from those observed in rats, suggesting that vascular sympathetic tone is similar in the species. HR responses to autonomic blockers, however, do differ between mice and rats. During daytime hours, when mice are resting, HR is generally higher than intrinsic HR, whereas in resting rats, values are usually lower or close to intrinsic HR (157). We estimated intrinsic HR as the resultant (±SD) value obtained by averaging HRs across several studies using combined muscarinic and β-blockade.

Fig. 5. Tracings illustrate the effects of an intravenous bolus injection of phenylephrine (A) and sodium nitroprusside (B) on MAP and HR in a conscious mouse. Note the biphasic response in MAP and HR following phenylephrine (indicated by arrows). For further explanations, see text. (Figure is redrawn from Ref. 88.)
[516 ± 74 beats/min, n = 5 (3, 52, 88, 124, 202)] or ganglionic blockade [503 ± 86 beats/min, n = 4 (88, 124, 173, 202)]. In rats, intrinsic HR is ~350 beats/min (157). In mice, resting values of HR are usually found in the range of 550 beats/min or higher. Therefore, it is not surprising that only limited tachycardic responses to atropine have been obtained, and profound reductions in HR (usually 150–200 beats) to both ganglionic or β-AR blockade have been reported. These data suggest that cardiac sympathetic tone is higher in the mouse species than in the rat. Noteworthy is the finding that, immediately following blockade of nitric oxide synthase (NOS) with N^G^-nitro-L-arginine methyl ester (L-NAME), HR drops to values as low as those obtained during β-AR blockade (88). In Fig. 6, we present data indicating that the bradycardic response to L-NAME is partly mediated by a direct effect on the sinoatrial node. After full cardiac autonomic blockade, HR decreases further when L-NAME is added. This suggests that NO has a direct stimulatory effect on sinoatrial firing. Evidence for this hypothesis has also been obtained in human studies (149). However, in the mouse, the effect of blockade of NOS on sinoatrial firing may be greater due to the specific anatomic location of the node. In contrast to the human situation, the sinoatrial node is located in the superior caval vein where it joins the right atrium rather than in the atrium itself (207, 218). Regarding the thin venous wall, it is therefore possible that vascular derived flow-dependent factors may directly influence the discharge rate of these nodal cells and hence influence HR. This concept is supported by the observation that endothelial NOS (eNOS)-knockout mice have lower resting HRs than wild-type controls (186, 190, 228). eNOS is expressed in cardiomyocytes of mice, but it is not required for normal autonomic control of cardiac function. In isolated cardiac tissue, chronotropic responses to muscarinic and β-AR agonists were not different between eNOS (−/−) and wild-type mice (205). If a central effect of NOS inhibition was involved in this response, then a tachycardia was expected. Recently, it has been shown that overexpression of eNOS in the nucleus of the solitary tract (NTS) causes bradycardia (177). Although neuronal NOS (nNOS) is colocalized with choline acetyltransferase in sinoatrial neurons (27), nNOS seems not important for the in vivo bradycardic effect of L-NAME, because nNOS-knockout mice have higher baseline HRs than wild-type controls (94) and are less responsive to vagal stimulation (27). In our view, these data suggest that under normal conditions, flow-induced NO production partly controls HR in the mouse.

### Assessment of Blood Pressure and HR VARIABILITY

Because of the sensitivity of blood pressure and HR to changes in autonomic nervous activity, spontaneously occurring variations in these parameters have been used to examine integrative autonomic control mechanisms in mice (52, 82, 88, 94, 95, 128, 190, 202, 214, 221).

### High-Frequency Oscillations

With the use of spectral analysis techniques, high-frequency (HF) variations of blood pressure and HR have been identified in the range of 2–8 Hz. The HF oscillations of pressure and HR are coupled to the respiratory cycle. In contrast to what has been found in humans, the amplitude of the HF oscillations of pressure and HR is not influenced by autonomic blocking agents (88, 95) but rather the result of mechanical disturbances caused by the respiratory cycle. The response time of the sinoatrial node and vascular smooth muscles is probably too slow to follow the respiratory-related fluctuations in autonomic nerve activity. Therefore, HF sympathetic nerve firing induces tonic effects on the target tissues rather than it contributes to fluctuations in their responses (89, 93).

### Low-Frequency Oscillations

In the so-called low-frequency (LF) band, i.e., below 1 Hz, sinoatrial and vascular smooth muscle cells are able to follow fluctuations in autonomic nerve firing with alternating activity. Accordingly, ganglionic or α-AR blockade decreases the amplitude of LF oscillations of blood pressure (88, 95). When sympathetic tone is increased, for instance during infusions of nitroprusside sodium (95) or lipopolysaccharide (LPS)-induced shock, LF oscillations, especially those occurring at 0.4
HZ, become a prominent feature in the blood pressure signal (Fig. 7). In rats, blood pressure oscillations at 0.4 Hz are caused by resonance phenomena in the baroreceptor reflex loop (93). The fact that the resonance frequency appears to be similar in rats and mice suggests that minimal time constants for the vascular component of the baroreceptor reflex loop are reached. Although muscarinergic blockade by atropine does not alter steady-state HR in mice, it reduces the amplitude of the LF fluctuations of HR (52, 88, 95). In contrast, β-AR blockade has either no effect or even enhances LF oscillations of HR. These data are discrepant with findings in humans, where β-AR blockade reduces LF HR variability (HRV). This is most likely explained by the difference in cardiac autonomic balance between the species. In mice, but not in humans, unmasking of the parasympathetic component by β-AR blockade increases HRV.

LF/HF RATIO

In humans, the ratio between LF/HF fluctuations in HR has been used as a marker of autonomic balance; LF for being predominantly influenced by sympathetic tone and HF for being mainly vagally controlled. Gehrmann et al. (52) proposed that this ratio could also be used to screen cardiac phenotypes in mice. Because HF oscillations are mainly mechanical of origin and atropine reduces LF HR variations rather than increasing them, the LF/HF ratio does not seem to be a valuable screening tool to assess autonomic balance in mice.

INFLUENCE OF EXPERIMENTAL CONDITIONS ON AUTONOMIC FUNCTION

Experimental conditions and environmental temperature are important factors that may influence the assessment of cardiovascular autonomic function in mice. For instance, Ishii et al. (82), using telemetry, compared HRs in voles (Microtus species) and body weight-matched laboratory mice. Voles live in cool environments without any form of hibernation, and their basal metabolism is ~20–40% greater than in laboratory mice as expected from allometric predictions. Assuming that heat production depends importantly on autonomic nervous activity, it was expected that HRs would be higher in the voles than in mice. Surprisingly, the opposite was found. Basal HRs were lower (~460 beats/min) in voles than in mice (~580 beats/min). In addition, tachycardic responses to atropine were larger in voles, whereas bradycardic responses to sympatholytic drugs were greater in the laboratory mice. At first glance, these results seem rather unexpected. However, the voles, obviously now being in a laboratory and warmer environment than normal, had probably less effort to maintain body temperature and therefore exhibited less sympathetic activation. In rats, raising ambient temperature to thermoneutral housing is known to reduce HR and blood pressure (158). This example illustrates the importance of environmental temperature for cardiovascular experiments in mice. Before surgical interventions, laboratory mice are normally housed in groups. After instrumentation, animals are often housed separately and have to spend more energy on maintaining body temperature. This may lead to an undesired increase of sympathetic tone and additional loss of body weight. In our experience, recovery from surgery is improved by keeping the mice at an ambient temperature of 30°C. When deprived of food, mice lose 10 to 20% of their body weight within 24 h. This is associated with a lowering of body temperature and may induce a hibernatinglike state called torpor (51).

AUTONOMIC CONTROL OF BLOOD PRESSURE IN GENETICALLY ALTERED MICE

Many transgenic and knockout mice have been generated that exhibit altered control of cardiac function and blood pressure. Here we will review the genotypes that show a potential deranged cardiovascular function because of genetic modifications in afferent (sensory), central (integrative), or efferent (effector) autonomic nerves.

GENETIC INTERVENTIONS THAT ALTER AFFERENT NERVOUS FUNCTION

Mechano-, chemo-, and osmoreceptors play a crucial role in autonomic feedback control mechanisms that maintain cardiovascular homeostasis. The molecular basis of these sensors is now emerging (42, 113, 212, 213). So-called epithelial Na⁺ channels (ENaCs) and the related acid-sensing ion channels mediate the translation of stimuli into intracellular signals in baroreceptor afferents and chemosensitive nerves, respectively. Vanilloid receptorlike proteins play a role in the cellular response of neurons of circumventricular organs to osmotic stimuli (113). These latter proteins

Fig. 7. Tracing of MAP obtained before (A) and 2 h after (B) intraperitoneal injection of lipopolysaccharide (LPS). At this time after LPS injection, blood pressure is maintained by sympathetic activation as can be observed from the predominant 2.5-s (0.4 Hz) oscillations that arise in the pressure signal. C: compares the average spectral power (±SE as indicated by the dotted lines) of blood pressure as obtained in 6 C57Bl6 mice.
are also involved in pain sensation (20). Genes that encode the proteins that form these channels have been cloned and knockout mice have been generated. In these genotypes, autonomic reflex functions have not been studied so far. Some studies explored the role of the ENaCs in the regulation of sodium by the kidney, where these channels are expressed too (163, 216). Remarkably, in α-ENaC heterozygous mice, blood pressure is more dependent on ANG II than in wild-type controls (216). This finding was interpreted such that the renin-angiotensin-aldosterone system was activated to compensate for the loss of ENaC proteins in the distal tubules of the kidney. An alternative explanation could be that ENaC defects in mechanosensation, similarly as after sinoaortic denervation, make blood pressure more labile and therefore more dependent on ANG II because pressure will fall more often under the threshold for renin release (160). Whatever the case, this example does illustrate the need for integrative studies in unraveling the in vivo function of such proteins.

Additional proteins that influence afferent nervous function have been genetically targeted. To these belong the neuropeptides calcitonin gene-related peptide (CGRP) and substance P (SP) (48, 78, 121, 156, 232), their receptors (16, 133), and enzymes that inactivate these neuropeptides such as neutral endopeptidases (NEP) (120, 181). SP and CGRP are not only important for the transmission of peripheral information to the central nervous system but also have efferent vascular functions. Both peptides are very potent vasodilators, and it has been suggested that the chronic release of these peptides contributes to arterial tonus and hence long-term control of blood pressure. The results of gene-knockout studies at these targets do not reveal a clear picture. To illustrate this, three examples are provided. Two research groups found that knockout mice for α-CGRP exhibited a substantial increase in blood pressure (48, 156). In contrast, blood pressures in α-CGRP-knockout mice studied by Lu et al. (121) were not different from those observed in wild-type controls. Second, the genetically induced loss of the primary receptor for SP, the neurokinin-1 (NK1) receptor, did not change basal blood pressure (16). Remarkably, this genotype was characterized by enhanced bradycardic responses to phenylephrine. This observation was rather surprising as, at least in other species, direct stimulation of NK1 receptors in the NTS induced potent depressor and bradycardic responses. Finally, in NEP-knockout mice, basal blood pressure was 20% lower than in wild-type controls (120). Because NEP also degrades bradykinin, this latter effect may be entirely due to accumulation of the latter vasodilating agent.

The abovementioned studies indicate that we are only about to begin to understand the molecular mechanisms of the sensory components of autonomic reflex loops that contribute to blood pressure control. When measuring components of such reflexes in closed-loop conditions, the missing gene product is not always phenotypically revealed and may even be (over)compensated by other factors (see the NK1 knockout study). Genetic deficits in afferent nervous function may be uncovered in particular conditions, by challenging the system. This concept is demonstrated in a recent study on the sensory nerve peptide synapsin (229). This peptide, which is normally involved in the regulation of neurotransmitter release at synapses, has also been located in microvesicles in sensory nerve endings, where its function is poorly understood. In synapsin-deficient mice, basal blood pressures and HR responses to nitroprusside were not altered. However, the blood pressure rise in response to cyclosporine A was greatly attenuated. As has been originally observed by Moss et al. (147), cyclosporine A raises blood pressure by sympathetic reflexes elicited by stimulation of afferent nerves, which was evidently blunted in the synapsin-deficient mice.

GENETIC INTERVENTIONS THAT ALTER CENTRAL NERVOUS FUNCTION

Many knockout and transgenic mouse models are available in which central nervous function is partially modified. These models are mainly used in the field of developmental and behavioral neuroscience and include, among others, mouse models with altered serotonergic, GABAergic, dopaminergic, or opioidergic functions (8, 19, 77, 152, 184). Because of their focus on higher brain functions, these studies generally lack a description of the cardiovascular phenotype and are, therefore, beyond the scope of this review. Many genetically modified mice models are used to pursue the role of the brain in controlling food intake and body weight (12, 75, 143, 164). However, despite the fact that the link to the cardiovascular phenotype is certainly relevant, concomitant hemodynamic measurements are infrequently performed in these studies. Application of telemetric techniques that provide simultaneously information on hemodynamic parameters may bring the abovementioned investigations closer to the cardiovascular research area.

The role of the brain renin-angiotensin system in blood pressure control through influencing volume homeostasis and sympathetic nervous activity has been widely recognized. But it is far from being understood. In the last decade, much insight in this area has come from studies using the transgenic TGR(mRen-2)27 hypertensive rat model, in which the Ren-2 mouse renin gene is inserted into the genome of Sprague-Dawley rats (148). Nowadays, many different mouse models have been developed in which the role of individual components of the brain renin-angiotensin system is being explored (1, 25, 37, 96, 145, 146, 176, 191, 225). Because this review focuses on the cholinergic and adrenergic mechanisms, we refer to these citations for further reading.

As advocated by Korner (107), control of cardiovascular homeostasis is not only restricted to phylogenetic older brain areas such as the medulla and hypothalamus. Cortical brain functions are involved, too, but they have received little attention up to now. The potential impact of such mechanisms is illustrated by a study in which it was found that genetic ablation of
fibroblast growth factor-2 was associated with impaired cerebral cortex development as well as impaired autonomic control of blood pressure (40, 41).

**GENETIC INTERVENTIONS THAT ALTER EFFERENT NERVOUS FUNCTION**

There is an abundance of mice with genetically induced alterations in efferent nervous functions. Many different targets have been modified. These include 1) nerve growth factors (66), neurotrophin-3 (NT3) (194), neurotutrin (74, 174), and transcription factors such as gata3 (116) that play a role in the maturation of the autonomic nervous system; 2) enzymes that are necessary for the synthesis and degradation of the natural transmitters acetylcholine (111, 224) and NE (26, 103, 165, 198, 199); 3) transporter proteins that mediate the uptake of neurotransmitters in cells (5, 168, 169, 185) or vesicles (83, 195); 4) a wide range of receptor subtypes for acetylcholine and NE; and 5) signaling proteins that mediate the final cellular response. Compared with the number of mouse models that is available to investigate the role of adrenergic nerves in the cardiovascular system, the number of mouse models for examining cholinergic function is relatively small.

**CHOLINERGIC NERVES**

*Nicotinic acetylcholine receptors.* Nicotinic acetylcholine receptors (nAchRs) mediate the fast synaptic transmission in the central nervous system as well as autonomic ganglia of the peripheral autonomic nerves. The nAchRs are pentameric receptors for which nine different subunits exist (34, 130). The distribution of these subunits may be specifically related to sympathetic and parasympathetic functions and varies regionally. On the basis of autonomic function studies that were performed in mice nullizygous for the ß7 subunit, it was suggested that ß7-nAchRs are specifically expressed in the sympathetic nervous system and mediate reflex-mediated increments in cardiac sympathetic tone (47). Whether these receptors are specifically coupled to this cardiac pathway is unknown. Identification of such specific locations and functions of nAchRs may provide new targets to control sympathetic outflow to specific vascular beds.

*Muscarinic acetylcholine receptors.* Muscarinic acetylcholine receptor subtypes may also be considered as targets to control autonomic function, mainly of the heart. Studies in knockout mice (57) confirmed the view that the M2 receptor is the predominant cardiac muscarinic receptor that, with stimulation, induces bradycardia and decreases inotropy. This is mediated by both Go and Gß proteins, because in mice lacking the ß1-subunit of the Go and Gß protein, muscarinic stimulation was unable to inhibit ß2-AR stimulation of L-type Ca2+ currents (24, 203). The myocardial content of Go proteins increases during hypertrophy and has been associated with ß-AR receptor desensitization (233). Baseline HRs in Go-knockout mice do not differ from wild-type controls and suggest that these proteins do not play a major role in nonpathological conditions (86). However, mice with reduced functional G protein ßγ-subunits have impaired parasympathetic control of HR (53). There are conflicting data in the literature whether M1 receptors control HR. Injection of the muscarinic agonist Mcn-A-343 increased HR in wild-type but not M1 receptor-knockout mice. This effect could be blocked by propranolol (64), which is consistent with the view that M1 receptors are present on the ganglia of cardiac sympathetic neurons and are able to mediate tachycardic responses. Other functions of mAchRs and their signaling proteins have been reviewed in detail by Caulfield and Birdsall (21).

**ADRENERGIC NERVES**

*Enzymes and transporters.* Cardiovascular phenotypes have been poorly described in mice models that lack genes encoding for enzymes and transporters that are involved in the synthesis and reuptake of catecholamines, respectively. Obviously, catecholamines are necessary for normal development. Many of these homozygous knockout models display severe sympathetic deficits and die perinatally (26, 103, 165, 195, 198, 199). Nullizygous genotypes may be rescued by feeding the dams synthetic precursors of catecholamines, such as L-threo-3,4-dihydroxyphenylserine (26, 198). Lack of enzymes that degrade catecholamines such as catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) is compatible with life. However, these models have been examined mainly in relation to behavioral and neurological studies (19, 77, 184). MAO-B- and COMT-knockout mice exhibit altered stress responses (56, 58). Cardiovascular phenotypes were not determined in these mice. However, given the strong association between psychosocial stress and cardiovascular abnormalities in mice (73, 217), such models may be of value to gain more insight into the molecular mechanisms linking behavioral and cardiovascular symptoms.

*Adrenergic receptors.* The molecular mechanisms mediating the effects of catecholamines on adrenergic receptors have been widely studied. The AR family consists of nine different receptors, namely three ß-ARs (ß1, ß2, ß3), three α2-ARs (α2A, α2B, α2C), and three α1-ARs (α1A, α1B, α1D). Pharmacological studies suggest the presence of a putative ß2-binding site (97). In accordance with their distribution pattern, α1-ARs have mainly been studied in relation to vascular function, whereas α2- and ß-ARs play a key role in central nervous/renal and cardiac function, respectively. For many of these receptors as well as their signaling proteins, knockout as well as transgenic mice are available. This is especially the case for ß-ARs and their signaling molecules, because of their predominant role in mediating cardiac hypertrophic diseases (43, 104, 114, 150, 162, 170, 171). Because of the prominent role of these receptors in mediating autonomic effects, these are discussed more extensively.
α1-ADRENERGIC RECEPTORS

α1A- and α1D-ARs, but not the α1B-ARs, are prime mediators of sympathetic arterial smooth muscle contraction (161). Despite this, α1-AR-knockout mice (all 3 subtypes) have normal baseline blood pressures (22, 155, 161), suggesting redundant vasoconstrictor activity by the other subtypes. Remarkably, overexpression of α1B-ARs was associated with hypotension (234). This was not due to impaired arterial contractile responses but rather related to reduced plasma catecholamine levels and depressed cardiac function (234). In addition to autonomic dysfunction, the transgenic phenotype also displayed Parkinsonianlike neurodegenerative abnormalities, which suggests that presynaptic mechanisms could be responsible for these symptoms (209).

α2-ADRENERGIC RECEPTORS

α2A-ARs. The α2A-AR is the most abundant receptor subtype in brain areas involved in cardiovascular regulation, and it mediates most of the classical sympathoinhibitory effects of α2-agonists (i.e., hypotension and bradycardia) (71). Mice lacking functional α2A-ARs have comparable (3) or slightly higher blood pressures (126, 127) than wild-type controls. In addition, resting HRs as well as plasma and cardiac NE concentrations were increased in these knockout mice, indicating that the loss of this receptor led to a hyperadrenergic state. The lack of an endogenous sympathetic feedback system may make the α2A-AR-knockout mouse also more susceptible to homeostatic challenges. Compatible with this view is the finding of Makaritsis et al. (126) that blood pressure rises quicker in α2A-AR (−/−) mice than in wild-type controls following salt loading. Besides a central sympathoinhibitory function, α2A-ARs exert an excitatory role in mediating peripheral local sympathetic sensory interactions. Kingery et al. (99) found that functional α2A-ARs, which are located on peripheral afferents (193), were required for the development of neuropathic heat hyperalgesia following peripheral nerve injury. Under normal conditions, the contribution of this latter mechanism to blood pressure control seems negligible because the lack of functional α2A-ARs is associated with slightly elevated blood pressures.

α2B-ARs. α2B-AR (−/−) and (+/−) mice have normal baseline blood pressures and HRs (118). However, these mice exhibit blunted transient blood pressure increases to α2-AR agonists, whereas hypotensive effects to the same agents are enhanced. These data suggest that α2B-ARs counteract the hypotensive actions of these agonists (71). This action is probably not restricted to vascular sites, but, given the relatively high density of α2B-ARs in the thalamus (197), also central sympathoexcitatory mechanisms should be implicated (125). This hypothesis is supported by the finding that acute hypertensive responses to an intravenous infusion of hypertonic saline were absent in anephric (to exclude renal effects) α2B-AR (+/−) mice, whereas in wild-type mice, pressure rose by 15 mmHg (127). This hypothesis is supported by recent findings in rats, in which local injection of antisense oligodeoxynucleotides targeted to α2B-ARs inhibited the induction of salt-induced hypertension (100). Thus the increased susceptibility of α2A-AR-knockout mice to homeostatic challenges may, in part, be mediated by compensatory actions of central α2B-ARs. Clearly, more research is needed to unravel the interactions between these receptors.

α2C-ARs. α2C-ARs seem of minor importance in autonomic control of blood pressure. α2C-AR-knockout mice display similar blood pressures and HRs as their wild-type controls during baseline conditions as well as during salt loading or application of α2-agonists (118, 125, 127). However, a recent study indicated a physiological role of this receptor subtype in cold-induced vasoconstriction in the cutaneous circulation. With the use of several selective α2A-AR antagonists, Chotani et al. (29) found that the increased expression of α2C-ARs may mediate the arterial constriction occurring in tail arteries during exposure to cold.

α2-ARs and imidazoline agonists. The sympatholytic effects in response to α2-AR agonists and imidazoline agonists have always been intimately coupled. Compared with the rat, the brain stem of the mouse is relatively enriched in imidazoline receptors (201). Peripheral as well as central administration of specific imidazoline receptor agonists decreases blood pressure in α2A-AR-knockout mice, at least in anesthetized conditions (201). However, in conscious conditions, hypotensive effects following peripheral administration of imidazoline agonists were not observed in mice carrying a point mutation in the α2A-AR (230). These latter data suggest that α2A-ARs are necessary for the cardiovascular effects of imidazoline agonists. As suggested by Head (67), α2-ARs may be located downstream of imidazoline-binding places in the sympathoinhibitory pathway.

β-ADRENERGIC RECEPTORS

β-ARs modulate a variety of homeostatic processes. The predominant subtype regulating HR and cardiac contractility is the β1-AR. The β2-AR subtype is mainly involved in mediating (vascular, uterine, and bronchial) smooth muscle relaxation, whereas the β3-AR subtype controls lipolysis in adipose tissue. Recently, β3-ARs have also been identified in the heart, where they oppose β1-AR function by inducing negative inotropic responses (50). The functional classification of β-AR subtypes is not absolute. Recent studies suggested that the β1-AR may play a dominant role in mediating vasodilatation in the mouse (30). In general, the functional classification appears well preserved across species (172).

Knockout models. Given the wide range of β-AR function in these autonomic processes and the unequivocal physiological effects of many β-AR antagonists, it is rather surprising that genetic ablation of β1-, β2-, or β3-ARs has no deleterious effects on cardiovascular and metabolic function. Resting blood pressures and HRs were not altered in β1-, β2-, or β3-, or
combined $\beta_1/\beta_2$-AR-knockout mice (31, 172, 173, 206). 
Deficiencies in $\beta$-AR function could be demonstrated when the cardiovascular system was challenged. 
Chronic reserve, as assessed with isoproterenol injections, was limited in $\beta_1$ (-/-) and $\beta_2/\beta_2$ (-/-) mice, but not $\beta_2$ (-/-) mice, suggesting that also in the mouse the $\beta_1$-AR is the dominant subtype controlling chronotropy. In $\beta_1$-AR-knockout mice, baroreflex function appears to be preserved by adapting parasympathetically nervous activity. After muscarinic receptor blockade, HR was lower in $\beta_1$-AR-knockout mice than in wild-type mice. In addition, chronotropic responses to sodium nitroprusside were almost totally blocked by atropine (173). These data indicate that the natural opposing role of the parasympathetic system compensates for the loss of $\beta_1$-AR function. Besides the parasympathetic system, $\beta_3$-AR may also constitute an opposing role to the excitatory function of $\beta_1$- and $\beta_2$-ARs. 

Varghese et al. (206) showed that in $\beta_3$ (-/-), inotropic responses to isoproterenol were greater than in wild-type controls. In wild-type mice, $\beta_3$-AR activation induces, via the $G_i$ pathway, the production of NO, which limits the inotropic response to isoproterenol. Recent evidence suggests that this mechanism may play a role in human heart failure, where $\beta_2$-ARs are upregulated and $\beta_1$-ARs are downregulated (144). However, the physiological relevance of this negative feedback mechanism needs further substantiation (50).

Models overexpressing $\beta_3$-ARs. In contrast to what was found in gene ablation studies, overexpression of $\beta$-ARs in the heart is associated with altered baseline cardiac function. In young mice, a fivefold cardiac-selective overexpression of $\beta_1$-ARs increases basal HR and contractile function (44). However, at advanced ages (>35 wk), the genotype is associated with marked hypertrophy and depressed cardiac function, supporting the view that increased $\beta_1$-AR signaling is ultimately detrimental (10, 44). Enhanced basal cardiac function was also found after cardiac-selective overexpression of $\beta_2$-ARs (137). However, the maladaptive effect of this intervention appears to be gene dose dependent. Liggett et al. (115) showed that mouse hearts tolerate the increased adrenergic drive caused by a 60-fold overexpression of $\beta_2$-AR, but they develop signs of failure when the level of overexpression of $\beta_2$-ARs is greater than 100-fold. Overexpressing $\beta_2$-ARs at 350-fold in the heart is associated with rapid and severe cardiac failure. Given the fact that $\beta_2$-ARs have a higher affinity for adenylyl cyclase than $\beta_1$-ARs, it is remarkable that much lower levels of overexpression of $\beta_1$-ARs than of $\beta_2$-ARs are required to induce cardiac dysfunction over time. Evidence for distinct signaling pathways in relation to apoptosis may contribute to this difference between $\beta_1$- and $\beta_2$-ARs (33, 231). Cardiac-specific overexpression of $\beta_2$-ARs is associated with reduced left ventricular function under baseline conditions. However, with stimulation with isoproterenol or the specific $\beta_3$-AR agonist L755507, cardiac function and adenylyl cyclase activity increased more in transgenic than wild-type mice (106). This indicates that the $\beta_3$-AR may have a dual role in the heart and couple to $G_\alpha$ proteins, too.

Models overexpressing $\beta$-AR-coupled signaling proteins. Increased adrenergic drive induced by overexpressing $G_\alpha$ proteins in the murine heart also elevates basal cardiac function and ultimately induces cardiomyopathy (84, 85). The maladaptive process in the $G_\alpha$-overexpressing mice can be blocked by $\beta$-AR blockade (4). However, not all genotypes that are characterized by a hyperdynamic cardiac state induce a cardiac pathological phenotype. Mice overexpressing a minipeptide inhibitor of $\beta$-AR kinase (105) or mice overexpressing a “cardiac” isoform of adenylyl cyclase (49, 175) do not have discernable histopathology. These data indicate that not all gene manipulations distal to the $\beta_1$-AR induce pathological changes. Therefore, additional signaling pathways must be involved. However, the presence of the endogenous agonists NE and epinephrine is essential in these processes (165).

Mice that overexpress $\beta_1$-ARs selectively in the atria have a normal life span and do not develop heart failure (128). Both $G_\alpha$-overexpressing mice as well as atrial overexpressing $\beta_1$-AR mice exhibit alterations in HRV. In these transgenic strains, HRV was depressed, which supports the view that enhanced adrenergic signaling saturates the sinoatrial myocytes and renders them less responsive to other stimuli (128, 202). In addition, the study in the atrial overexpressing $\beta_1$-AR mice shows that depressed HRV per se does not limit life span. It also signifies that autonomic function parameters such as HRV may have prognostic value only in diseased states.

In contrast to these hyperdynamic models, Dash et al. (35) recently explored whether inhibition of the $\beta$-AR signaling cascade would trigger a reactive increase in cardiac sympathetic tone to compensate the imposed inhibitory drive. To this end, they created mice with a fourfold overexpression of phospholamban (PLB). In young PLB transgenic mice, ventricular function was only mildly depressed and associated with elevated plasma cathecholamine levels and an elevated phosphorylation status of PLB, suggesting endogenous sympathetic compensation. However, at advanced ages, the compensation progressed into decompensation and was associated with desensitized adenyyl cyclase, impaired responses to isoproterenol, heart failure, and premature death. This “reactive” model mimics characteristics of human heart failure better than the direct “forward” models. The nature of the feedback loop between PLB and sympathetic hyperactivity needs to be further explored.

CONCLUSIONS

The available evidence suggests that in the mouse, unlike in humans, the autonomic balance is heavily dominated by the sympathetic nervous system and that parasympathetic contributions are only minor. Despite the sympathetic predominance, studies from genetically modified animals have indicated that further enhancement of central sympathetic outflow is
associated with hypertension in mice. More specifically, raising cardiac efferent sympathetic activity in the mouse increases the susceptibility for developing hypertrophic cardiac diseases. Thus, despite altered basal autonomic balance, genetic mouse models appear to be useful tools to explore molecular mechanisms relating increased sympathetic activity to human cardiovascular pathologies. Future studies in such models may identify new targets that could enable regionally selective interventions in autonomic nervous activity. To these targets belong not only organ-specific postsynaptic signaling mechanisms but also sensory, central, or presynaptic components that, in these closed-loop control systems, contribute to the initiation or maintenance of a diseased state. These investigations will certainly increase our insight into how the autonomic nervous system contributes to homeostasis in a widely varying genetic background.

Because hemodynamics in the mouse are variable and sensitive to environmental disturbances, there is need to standardize recording conditions to allow comparison of hemodynamic parameters between studies. Measurements in conscious conditions are complex but may prove to provide more sensitive traits for detecting genetically induced modifications in cardiovascular control, which are not associated with obvious histopathological defects or require homeostatic challenges to demonstrate a particular genetic deficiency. A further refinement may be achieved by applying mathematical techniques that quantify the dynamic variation of hemodynamic parameters as well as by improving technology, for instance, by telemetry (regional) blood flow measurements.

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REFERENCES


63. Hallemeesch MM, Ten Have GA, and Deutz NE. Guyton AC and Hall JE.

64. Hamilton SE, Hardouin SN, Anagnostaras SG, Murphy PA, and Kobilka BK. Hein L, Limbird LE, Eglen RM, and Kobilka BK.


100. Kintsurashvili E, Gavras I, Johns C, and Gavras H. Effects of antisense oligodeoxynucleotide targeting of the α(2B)-


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