Local adenosine concentrations contribute to the signal-

Twenty years ago it was postulated that changes in secretion rate if tubular flow rate falls and vice versa. A decrease in vascular tone and an increase in renin changes in proximal tubular flow rate to afferent arteriole to form the juxtaglomerular apparatus. Within this region, the TGF communicates afferent arteriole to form the juxtaglomerular apparatus, completely lacking tumor necrosis factor-α (TNF-α) display threefold elevated renal renin mRNA levels under control conditions as well as after salt depletion provides the first in vivo evidence for a possible physiological function of TNF-α as a negative regulator of renin synthesis (35). Studies in TNF-α −/− mice also indicate that a strong increase in the local production of TNF-α initiates intimal hyperplasia after vascular injury (38).

This example illustrates the major strength of the genetic approach compared with the more classical pharmacological and correlative approaches. Although the latter are inherently indirect, pharmacological studies often suffer from uncertain pharmacokinetics and a lack of specificity. Accordingly, the observation of a sevenfold higher salt intake after an overnight fluid restriction in mice carrying a targeted deletion of the oxytocin gene constitutes strong direct evidence for the concept derived from infusion studies of agonists and antagonists that sodium appetite is negatively regulated by central oxytocin (1). The finding that mice lacking tumor necrosis factor-α (TNF-α) display threefold elevated renal renin mRNA levels under control conditions as well as after salt depletion provides the first in vivo evidence for a possible physiological function of TNF-α as a negative regulator of renin synthesis (35). Studies in TNF-α −/− mice also indicate that a strong increase in the local production of TNF-α initiates intimal hyperplasia after vascular injury (38).

Further examples for important advances in our understanding of cardiovascular regulation obtained by the use of gene-targeted mice are the discovery of a major role of the K⁺ channel β-subunit KCNE1 in K⁺ and renal fluid homeostasis (36), the unmasking of an enhanced central response to dehydration in angiotensin AT₁A −/− mice (24), the identification of multiple physiological functions of α₂-adrenergic receptors (27),

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in focus

Mouse gene targeting in cardiovascular physiology

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In the 1980s a revolution took place in cardiovascular physiology unnoticed by many researchers working in the field. At that time, the rat and the dog were the prime animal models to study integrative mechanisms involved in cardiovascular regulation. The notorious but true saying that cells do not have a blood pressure implies that in vivo experiments will always be critical in the process of discovering new principles of circulatory control. Studies in rats and dogs have helped to unravel many basic principles of cardiovascular physiology, such as autoregulation of blood flow (11, 28), reflex control of blood pressure (3), or blood volume regulation through signals originating from the heart (2), to name just a few. Nevertheless, the lack of techniques to directly link the activity of specific genes to physiological functions—which was then possible already in cellular systems—remained a major drawback. Thus the development of experimental procedures to induce mutations in single genes and the eventual successful generation of mice carrying a targeted mutation in 1989 (7, 19) marked the beginning of a new era area also in cardiovascular physiology.

Today, more than 3,000 different knockout mice have been constructed. Many of these mutations affect cardiovascular function, and some of them have helped to solve long-standing open questions in cardiovascular regulation. For example, it has been known for long that the tubuloglomerular feedback (TGF) is a key mechanism of autoregulation of renal blood flow and glomerular filtration and is intimately involved in blood volume homeostasis. In the kidney, the proximal part of the distal tubule gets in close contact with the afferent arteriole to form the juxtaglomerular apparatus. Within this region, the TGF communicates changes in proximal tubular flow rate to afferent arteriolar smooth muscle and renin-secreting cells, causing a decrease in vascular tone and an increase in renin secretion rate if tubular flow rate falls and vice versa. Twenty years ago it was postulated that changes in local adenosine concentrations contribute to the signal-

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and the demonstration that the vagal effects on atrial rate are independent from M₃-muscarinic receptor signaling (33).

In accordance with its increasing importance in cardiovascular research, the basic physiology of the murine circulatory system has been intensively studied recently. Most relevant techniques and experimental protocols to assess cardiovascular function have now been adapted to the small size of the mouse. These include chronic measurements of blood pressure (6, 14, 21, 23) and cardiac output (16), the analysis of the different components of the baroreceptor reflex (22), and renal function and balance studies (21). Many aspects of the murine cardiovascular system—anatomic and physiological—are identical to larger mammals. Nearly all values of cardiovascular parameters obtained in mice are well within the range predicted by allometric scaling equations from rats and humans (15). The baroreceptor reflex (22) and the regulation of fluid and electrolyte homeostasis by the renin-angiotensin system (8), two of the major blood pressure regulating systems (30, 32), seem to function in a very similar fashion as in larger mammals.

Nevertheless, there are also important peculiarities specific to the mouse. For example, studies using autonomic blocking agents indicate that mice generate a high resting cardiac sympathetic tone (14, 17). Mice have a relatively labile blood pressure and are extremely sensitive to stressful conditions such as anesthesia (21) and changes in ambient temperature (37). Sympathetic neurotransmitter release is also enhanced by stressful housing conditions, such as social deprivation and exposure to novel odors (9). A negative energy balance can cause severe hypotension and bradycardia in mice, possibly resulting in torporlike states (37). The development of hypotension is accelerated if mice are housed at an ambient temperature of 23°C, which is the normal room temperature in most laboratories. Dietary preferences (31) as well as metabolic responses to leptin (13) differ between strains and can confound cardiovascular measurements. The metabolism of melatonin is also strain dependent (18), which may impact on circadian cardiovascular rhythms. These factors need to be considered when designing experiments in mice.

It is very likely that targeted deletions will be introduced into most of the mouse’s ∼30,000 genes within the next few years. Keeping in mind the murine peculiarities listed above, the combination of mouse genetics and physiology seems to have a bright future. There are, however, several caveats. Most importantly, the vast majority of targeted deletions inactivate the target gene in each cell from conception onward. Such a lifelong deletion of gene function cannot only severely alter developmental processes, but might also disturb the normal homeostatic balance, causing secondary and/or compensatory up- and downregulation at the level of the genome as well as the level of the organism. Thus more subtle genetic constructs will be required, such as conditional targeted deletions and combinations of gene inactivations with the introduction of transgenes that restrict the expression of a specific gene to a limited area (e.g., in the central nervous system) or time (e.g., during development) (10, 20). Furthermore, although gene targeting is a very efficient way to investigate the function of a certain gene, it is considerably less powerful to uncover the genetic pathways underlying a given physiological process, particularly if the biology of this process is only poorly understood. In this latter situation, mutagenesis screens are much more likely to yield new insights. Such screens, however, require huge numbers of animals to be generated, housed, and phenotyped. Even though mutagenesis screens are already performed in mice, they can be realized much easier in the zebrafish (4, 26). And, finally, despite all the similarities between mice, rat, and humans, we will always need research that translates the results obtained in mice to the human physiology and pathophysiology (12).

REFERENCES

14. Janssen BJA, Leenders PJ, and Smits JFM. Short-term and long-term blood pressure and heart rate variability in the