In complex systems such as blood pressure homeostasis, physiological compensatory mechanisms effectively mask any perturbations to the status quo. When these mechanisms fail to restore homeostasis, the presenting phenotype may be far removed from the initial perturbation, making it difficult or even impossible to unravel the underlying etiology. This is the case with hypertensive rat models such as the spontaneously hypertensive rat (SHR), where a vast body of knowledge has been accumulated, but progress has been slow in identifying the true underlying cause or causes of hypertension.

Instead of establishing genetic models by phenotypic selection, it is possible via transgenesis to make phenotypic models through the use of selected genes or alleles. However, in all but the very simplest transgenic manipulations, it still may be difficult to distinguish "cause" from "effect." When single genetic alterations are introduced into the germline, the researcher at least has a clear starting point from which to unravel the phenotypic response. Such is the case with the TGR (mRen-2)27 transgenic rat, which was generated by the stable introduction of the mouse Ren-2 renin gene into the rat germline, with the resulting phenotype of elevated blood pressure.

This transgenic strain was constructed with three specific aims in mind, namely, (1) to establish transgenic technology in this species, giving greater flexibility for genetic experimentation in areas such as cardiovascular research, neurobiology, and physiology; (2) to directly test the ability of the granular convoluted tubule cells of the rat submandibular gland (which do not express endogenous rat renin) to express the mouse Ren-2 gene, a gene expressed at high levels in mouse granular convoluted tubule cells; and (3) to examine whether the Ren-2 enzyme has activity in vivo and could affect blood pressure per se. Although it was not specifically designed to model essential or any other form of human hypertension, this rat strain has the potential to yield insight into the mechanisms whereby apparent low plasma renin levels can be associated with hypertension.

The initial findings that plasma and kidney renin levels were normal to low suggested negative feedback on the endocrine renin-angiotensin system (RAS), and in situ hybridization of transgenic kidneys, together with immunohistochemical analysis, indicated that renal renin expression was strongly suppressed. Paradoxically, plasma prorenin levels were extremely elevated. Blood pressure could be effectively reduced by converting enzyme inhibition or by an angiotensin II (Ang II) receptor antagonist, a finding not inconsistent with the involvement of Ang II in the development of hypertension in the transgenic animals. In view of the apparent lack of kidney involvement in the generation of hypertension in these rats and the demonstration that the majority of plasma renin and prorenin was transgene derived, the possibility of extrarenal renin activity was proposed. An obvious candidate for a tissue RAS system was the adrenal gland, because this tissue exhibited the highest expression of the transgene (followed by the thymus, tissues of the gastrointestinal and genital tracts, kidney, brain, and lung; no expression was observed in the liver or submandibular gland) and this organ has been the subject of intensive study in the TGR(mRen-2)27 strain. In situ hybridization, together with RNase protection analyses, revealed enhanced expression of the transgene in both the zona glomerulosa and zona fasciculata, and this was confirmed by direct measurement of renin and prorenin levels in the capsular and decapsular portions of the TGR adrenal. The site of expression within the adrenal gland indicates interplay between the cis-acting sequences of the mouse Ren-2 transgene and trans-acting factors specific to the rat adrenal cortex, because the tissue distribution of expression is more similar to that of endogenous rat renin within the adrenal than to the expression of the Ren-2 gene in the mouse adrenal gland, which is normally limited to the zona fasciculata and the X zone. (In the normal Sprague-Dawley rat, low levels of active renin are limited to the zona glomerulosa.) In vitro studies of adrenal cell suspensions confirmed that large amounts of Ren-2 renin and prorenin were being produced by adrenal cortical cells, and adrenalectomy studies confirmed the role of this organ as a source of prorenin in vivo. After bilateral adrenalectomy, prorenin levels were dramatically decreased (although not to normal levels). Blood pressure was also decreased significantly, but this could have been due to the lack of mineralocorticoids after adrenalectomy. Urinary glucocorticoid and mineralocorticoid excretion is significantly enhanced in the transgenic rats up to 18 weeks, suggesting adrenal stimulation of steroid metabolism, but the mineralocorticoid receptor antagonist spironolactone has no effect on blood pressure. Zonal distribution of aldosterone is also altered, with substantial amounts being found in the zona fasciculata as well as the zona glomerulosa, to which it is normally limited. Partly as a result of such findings, a reevaluation of renin expression in normal and abnormal human adrenal tissues is presently taking place.
A number of possible hypotheses as to the mechanism of hypertension are under investigation in different laboratories. Tokita et al describe the detailed analysis of renin activity in the TGR(mRen-2)27 strain. As previously described by Poulsen and Jacobsen, the kinetics of rat angiotensinogen cleavage differ, depending on whether mouse submandibular gland renin or rat renin is used. From biochemical studies of the TGR(mRen-2)27 rat, Tokita et al provide evidence that the altered kinetic parameters of the mouse renin/rat angiotensinogen interaction may contribute to the development of hypertension in this model. The authors confirm the results of Peters et al and Yamaguchi et al demonstrating that the majority of plasma renin is transgene derived. The difference in pH profile between mouse Ren-2 renin and rat renin acting on rat angiotensinogen will certainly be reflected in in vitro assays of renin activity, but its significance in vivo is unclear. The authors demonstrate that "a little mouse renin goes a long way," but that is clearly not the whole explanation. The potential for increased angiotensinogen cleavage is not reflected in a significant increase in Ang I or Ang II levels in the Ang II circulation, and this adds weight to the suggestion that tissue RAS may play an important role. Indeed, experiments using isolated perfused hindquarter preparations suggest that the transgenic rats exhibit increased vascular angiotensin formation, which is unaffected by bilateral nephrectomy. Also, Ren-2 messenger RNA has been found in mesenteric and aortic tissue of transgenic animals, confirming its expression in vascular tissue. Comparison between the TGR(mRen-2)27 rat and transgenic mice expressing rat angiotensinogen is difficult, because no information is available concerning the kinetics of rat angiotensinogen cleavage by mouse Ren-2 renin, the isozyme present in these transgenic mice.

On a more general note, Tokita et al highlight the fact that renin activity measurements require standardization, because interlaboratory comparisons are difficult unless detailed assay conditions are reported. Even the term plasma renin concentration is misleading because "plasma renin concentration" and "concentration of renin" mean quite different things. It is perhaps time that terminology was brought in line with standard biochemical practice.

In general, homozygous TGR(mRen-2)27 rats require antihypertensive treatment to be successfully maintained much beyond 6 weeks of age because of their significantly higher mortality. The use of homozygote animals by Tokita and colleagues may suggest selection of surviving animals or differences in diet.

Because of the fact that the Ren-2 rat is not totally inbred, the establishment of multiple colonies in different centers will inevitably result in the generation of TGR(mRen-2)27 substrains with slightly different genotypes and probably slightly differing phenotypes. It will be increasingly important, both now and for future transgenic experiments, that a strain designation (e.g., the abbreviated name of the institute breeding the animals) be used to aid in the comparison of data between laboratories. Indeed, we have strong evidence (J.J.M., unpublished observations) that the source of Sprague-Dawley rat on which the transgenic strain is maintained may have a dramatic effect on penetrance of the phenotype. The introduction of the Ren-2 transgene directly onto an inbred background should provide a stable genetic background, with coisogenic control animals readily available.

The Ren-2 transgenic rat strain has the advantage of being the result of a single genetic change. The fact that this change involves the introduction of DNA from another species does not lessen the demonstration that blood pressure can be heritably altered by a single genetic change. This model provides a strain that has a highly active extrarenal RAS and demonstrates the feasibility, through the selective manipulation of the endogenous RAS, of informative experiments to ascertain the function of nonrenal RAS in detail. Although the Ren-2 strain accomplished the original experimental objectives, it has limitations as a model. First, for practical reasons at the time, the original transgenic founder was not generated on an inbred background, so there is no true genetic control for the model; the Hannover Sprague-Dawley strain is probably the best alternative. Second, by its nature, the model uses a mouse renin gene, and although on the one hand the differences between the DNA, mRNA, and protein sequences can be elegantly exploited experimentally, the fact that it is a heterologous transgene brings with it limitations.

While new transgenic rat strains continue to address specific questions, it is important to place the Ren-2 rat in perspective (Figure). Inevitably, this strain has been described as "unusual" and even "inappropriate" as a model of human hypertension. However, it is a significant milestone in cardiovascular research and opens the way to the generation of a whole range of transgenic animals designed to answer specific questions posed not only by TGR(mRen-2)27 but by studies of classic genetic models of hypertension. The ability to manipulate the rat germline makes it possible to study individual genes in isolation and to correlate their expression with physiological phenotypes. However, to clarify the contribution made by many candidate genes, transgenic rat technology needs to be further developed such that homologous recombination becomes a viable option. This will require the elaboration of appropriate embryonic stem (ES) cell lines.

Although the production of ES cell lines from strains such as the SHR would provide the most powerful experimental possibilities, it is likely that rat ES cells will be produced first from a traditional laboratory.
strain. To date, a number of groups have attempted to isolate rat ES cells with no reported success despite the generation of several candidate cell lines. True ES cells must not only be shown to generate chimeras but also to be capable of germline transmission. This is a daunting if not impossible task at present, but the availability of such lines would catapult rat molecular genetics to new heights and must be one of the major goals in experimental hypertension research during the coming years.

The introduction of transgenic rat technology has provided new possibilities for basic hypertension research. The relatively rapid elucidation of transgenic strains as a consequence of their relatively simple genetic manipulation provides the key to the use of transgenesis in hypertension. With the use of commercial inbred strains as transgenic hosts, it will become easier for researchers to exchange stocks without concern over appropriate control strains. The genetic characterization of traditional genetic models of hypertension using methods such as microsatellite and minisatellite mapping is moving ever closer to the identification of the genes contributing to the hypertension. Such candidates can be tested by direct introduction into the rat germline. These two strategies are therefore complementary and will eventually form a continuous approach as both genetic mapping studies and gene manipulation techniques are further refined. This will undoubtedly lead to a better understanding of essential hypertension.

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