Transgenic laboratory animals in hypertension research

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Introduction

Over the last few years research has been focussing increasingly on the investigation of the pathophysiological basis of hypertension. It has become obvious that hypertension is a disease with an important genetic background. However, it is not yet clear which genes are involved. Animal models such as spontaneously hypertensive rats (SHR) are useful models for primary hypertension. In these animals hypertension is a polygenic trait, in which both autosomal and sex-linked genes can influence blood pressure (Lindpaintner at al., 1990). In linkage studies of crosses between hypertensive and normotensive rats, Hilbert et al. (1991) and Jacob et al. (1991) localized two gene loci that contribute significantly to blood pressure variation in these strains. In addition, there are several candidate genes considered to play an important role in the pathogenesis of primary hypertension, components of vasoactive regulatory systems.

The possibility to modulate gene expression and phenotypic influences of a defined candidate gene in intact animals by transgenic techniques provides a powerful tool to analyze the contribution of this gene to the physiology or pathophysiology of diseases such as primary hypertension. These transgenic changes can also be induced in a tissue-specific manner to study functional aspects of organ- or cellspecific promoter function. It can be concluded, that transgenic techniques are helpful to investigate problems that are closely related to the pathophysiology of primary hypertension.

In the past decade several genes have been cloned and extensively analyzed which code for hormones playing an important role in blood pressure regulation. These include genes of the renin-angiotensin-system (RAS), the adrenergic system, the endothelinsystem, nitric oxide system, and others. Some of these genes have been investigated in sufficient detail so that they can be used for in vivo transfer of genomic fragments into animals.The resulting transgenic animals then could become valuable models for research in primary hypertension.

Until now, transgenic techniques have been developed for many different species, and transgenic mice have been used most extensively. In hypertension research, however, rat models have been proven to be more useful than mice. Rats can be investigated very easily by using methods of whole animal physiology and pharmacology. In addition there are already many genetically hypertensive rat strains such as the SHR existing which could be easily used as reference strains.

As of this writing there are several techniques used to produce transgenic animals:

- retroviral infection of preimplantative blastocysts (Hogan et al., 1986; Jaenisch et al., 1981, Soriano et al., 1986)
- use of embryonic stem cells previously transfected with the DNA of interest to obtain organisms via microinjection into blastocysts (Evans, 1989; Robertson, 1991; Williams, 1990)
- direct microinjection of foreign DNA into the pronucleus of fertilized oocytes (Babinet et al., 1989; Gordon et al., 1983; Pattengale et al., 1989)

Microinjection of foreign DNA into the pronucleus of fertilized oocytes is used by most groups developing transgenic animals because this method is well established and extensively used. Recently, this method has been applied to the development of transgenic rats (Hammer et al., 1990; Mullins et al., 1990), whereas transgenic mice models have been available for more than ten years. For detailed information on the making of transgenic animals see: Brinster et al., 1985 (mice); Gordon et al., 1983 (mice); Paul et al., 1992 & 1994 (rat).

Transgenic rats in hypertension research

At present there have been few transgenic rat strains established which express candidate genes for hypertension. In these animals genes of the renin-angiotensin have been successfully integrated. There are several reasons why components of the renin-angiotensin-system have been used as gene constructs (for review see Ganten et al., 1991). For example there is linkage between human angiotensinogen gene mutations and hypertension. A genetic polymorphism in the human angiotensinogen, resulting in significantly different plasma levels and hypertension, is assumed to be a genetically linked predisposition for essential hypertension (Jeunemaitre et al., 1992). Apparently the human angiotensin-converting-enzyme is not directly related to hypertension in humans, but associated with myocardial infarction (Cambien et al., 1992) and cardiac hypertrophy (Schunkert et al., 1994). Higher plasma levels of this enzyme have been found associated with a deletion polymorphism of the gene. The homozygous genotype for this polymorphism is assumed to be accompanied by a higher risk for myocardial infarction apart from other risk factors. It is, therefore, possible that the RAS is not only involved in the development of hypertension, but also in related disorders as cardiovascular end-organ damage. The renin gene has also been linked to hypertension in animals. A restriction length polymorphism (RFLP) of this gene in Dahl salt-sensitive rats is linked to hypertension. In contrast, there is no such linkage in humans (Naftilan et al., 1989). All of the candidate genes for hypertension mentioned above have been used to develop transgenic animals to study the resulting gene effects in vivo. Initially, a number of transgenic mouse lines, expressing genes of the RAS, were developed, but now rats are the predominant species to be used for transgenic experiments in hypertension research.

The Hypertensive Rat TGR(mRen2)27

The first DNA construct which was used for developing an transgenic rat strain in hypertension research was the mouse Ren-2 gene. A genomic construct was directly injected into oocytes of normotensive rats (Mullins et al., 1990). The transgenic animals exhibited fulminant hypertension. The identical genomic construct had already been used to develop transgenic mice (Mullins et al., 1989). Three transgenic lines were generated [TGR(mREN)25,26,27] and further characterized. Up to now, the phenotype of line 27 has been investigated extensively. After 5 weeks of age, male heterocygous animals develop hypertension shortly after weaning. At the age of 10 weeks there is a plateau of blood pressure (about 240 mmHg) (Mullins et al., 1991). Homozygous rats achieve even higher blood pressure values of up to 300 mmHg and would die early, due to cardiovascular consequences, if they were not treaten with ACE inhibitors or other antihypertensive substances (Ganten et al., 1991). As in humans, female rats are far less hypertensive (up to 60 mmHg) than male animals. Androgens and their effect on the RAS seem to cause this sexual dimorphism. Treatment of female rats with dihydrotestosterone significantly elevates blood pressure, whereas orchiectomy has the opposite effect (Bader et al., 1992). Tissue-specific expression of the transgene revealed to be highest in the adrenal gland, whereas the kidney expressed only low levels, most probably due to feedback inhibition by the high blood pressure (Peters et

al., 1992). Protein concentrations of tissue renin confirm the mRNA measurements, being high in the adrenal gland and low in the kidney. Since ACE inhibitors or ANG II antagonists cause lower blood pressure in TGR(mREN2)27 animals, hypertension is dependent on ANG II. Active renin concentration and nearly all other parameters of the RAS, like ANG I, ANG II and angiotensinogen in the plasma of TGR(mREN2)27 are not elevated in heterozygous control animals, whereas prorenin, which is mainly derived from the adrenal gland, is greatly enriched. After bilateral adrenalectomy, prorenin levels are reduced to 20% (Sander et al., 1992) and blood pressure is lowered nearly to normotensive values. The physiological relevance of the high prorenin levels is unclear at the moment and therefore investigated. The significance of the adrenal gland in the pathogenesis of hypertension in these animals was further investigated by measuring the coricosteroid production. During the development of hypertension in young TGR(mREN2)27 animals, urinary excretion of deoxycorticosterone, corticosterone, and aldosterone was significantly elevated, when compared to control rats (Sander et al., 1992a). Treatment with ACTH enhanced the urinary corticosterone excretion more than in Sprague-Dawley control rats. These data indicate that there is a close relationship between ACTH, the intra-adrenal RAS, and steroid production by the adrenocortical cells in TGR(mREN2)27. Treatment with dexamethasone significantly lowered blood pressure in these animals (Djavidani et al., 1992), whereas spironolactone did not reduce blood pressure (Bachmann et al., 1992). Hypertension in these animals probably is not dependent on mineralcorticoids, whereas there might be an interaction between increased renin and production of adrenal steroids.

Recently, Hilgers et al. (1992) started to investigate the cardiovascular system and could demonstrate that the vasculature of the transgenic animals produced increased amounts of ANG II most likely being the result of the overexpression of the transgene. In addition, it was shown by RNAse protection assay that the Ren-2 expression was elevated in blood vessels. The fulminant systemic hypertension of TGR(mRen2)27 leads to pathological changes including hypertrophy and fibrosis in the heart (Bachmann et al.,1992). Current studies investigate the cardiac function and its cellular basis in more detaile.

Tepel et al., (1994 and 1994a) described that there is an increased cytosolic sodium and reduced Na+/K+-ATPase activity and reduced sodium-proton exchange activity in lymphocytes from transgenic rats.

In addition Böhm et al. (1994) described cardiac hypertrophy due to β-adrenergic neuroeffector mechanisms.

Transgenic Animals Expressing the Human Renin Gene

Fukamizu et al. (1991) generated transgenic mice using the human renin gene. The transgene in these animals was detected in the kidney and in other cardiovascular organs. The transcript was correctly spliced and the resulting protein was confined to juxtagolmerular cells as detected by human-specific anti-renin antibody.

A construct containing the human renin gene (17.6 kb construct after stripping it from vector-encoded sequence) was microinjected into oocytes from Sprague-Dawley/WKY hybrids (Ganten et al., 1992). Two of these founder animals transmitted the transgene to their offsprings. Active human renin was produced and secreted into the plasma by these animals, as determined by immunoradiometric assay with specific monoclonal antibodies for human renin. The plasma activity of renin was 6-fold increased compared to renin in human plasma. The rat prorenin, renin, ANG I, ANG II, angiotensinogen levels were compared to control animals, unaffected by the human renin. This indicates that the human renin does not react with rat

angiotensinogen to produce angiotensins. In addition, both strains of transgenic animals were normotensive.
Sodium depletion in these animals for three days caused a 11-fold increase in active human renin level, as to those of rat renin. These results demonstrate that the transgene is regulated by sodium-depletion and does not interfere with rat renin production under stimulated conditions.

Transgenic Animals Expressing the Human Angiotensinogen Gene
Several transgenic rats and mice were generated using a genomic human antiotensinogen gene construct (Ganten et al., 1992; Ohkubo et al., 1990). In transgenic mice, mRNA-expression could be mainly detected in the liver, but also in other organs such as brain, kidney, and heart (Ganten et al., 1992). The four rat lines being transgenic for human angiotensinogen gene showed increased but variing levels of angiotensinogen from 120g/ml up to 5 mg/ml. These levels exceed the level in humans, which is approximately 60 g/ml. Although they had high levels of angiotensinogen, these animals were not hypertensive, which demonstrates that human angiotensinogen does not interact with rat renin at physiological concentrations (Ganten et al., 1992). Transgene positive and transgene negative rats had the same level of angiotensinogen and angiotensin II. The transgene expression was highest in liver and ten-fold lower in the kidney and the gastrointestinal tract. Transgenic mice carrying the human angiotensinogen gene showed different expression patterns. The expression of the transgene in the kidney was as high as in the liver in contrast to humans, where kidney angiotensinogen is low (Takahashi et al., 1991). In crossbred transgenic animals, which express both angiotensinogen and renin genes, the possibility of local ANG I production is possible due to interaction of the human proteins and the functional role of tissue-specific RAS should be investigated.

Species-specificity of the Human Renin Substrate Reaction in Transgenic Animals
Rats carrying human angiotensinogen or renin genes remained normotensive, which is an indication of the species-specifity of these genes. Human renin did not interact with rat angiotensinogen, nor did human angiotensinogen with rat renin. The ANG II level in rats was unaffected. Blood pressure did not increase when the human-specific renin inhibitor, Ro 42-5892 was given as a bolus injection to human renin transgenic animals. An ANG II receptor antagonist lowered blood pressure significantly. Injections of recombinant human renin to rats being transgenic for human angiotensinogen, however, caused blood pressure increase of up to 50 mmHg. Injections of Ro 42-5892 immediately normalized blood pressure. There was no increase of blood pressure in control rats, which demonstrates that this blood pressure response was due to the interaction of human renin and angiotensinogen.

Future Perspectives of Hypertension Research in Transgenic Rats
In the next years hypertension research with transgenic rats and mice will focus on new candidate genes for essential hypertension. Several candidate genes are already established in transgenic mice models. The information obtained from these models can now be applied for the rat model. In the following, some of the gene targets are reviewed briefly.

Atrial natriuretic peptide (ANP) is a peptide hormone synthesized mainly by atrial cardiomyocytes. When injected into a rat, ANP has a natriuretic and diuretic effect, as well as it reduces arterial blood pressure. To study the effects of ANP on the cardiovascular system Seidman et al. (1984) introduced the ANP promoter into a mouse model. Steinhelper et al. (1990) developed a transgenic mouse harbouring mouse ANP. These transgenic mice developed chronically evelated plasma ANP

levels and had significantly lower arterial blood pressure compared with non-transgenic control animals.

Another important target for hypertension research is the endothelin peptide family and its receptors. Up to now, these substances have been characterized extensively by biochemical, pharmacological, physiological and molecular biologic methods, but the role of endothelin remains still uncertain. Recently Paul et al. (1992) reported the development of an endothelin-2 positive transgenic rat model, which might be a powerful tool to investigate the regulation and function of endothelin.

An additional gene of interest is the human heart chymase (Urata et al., 1990), which is a highly specific angiotensin II-forming enyzme in the human heart. This substance is not inhibited by ACE inhibitors. It has been hypothesized that ACE inhibitors only work effectively in the atrium because ACE is expressed higher there than in the ventricles. Therefore, chymase which is highly expressed in ventricles may not be blocked in the ventricles by usual ACE inhibitor therapy and, therefore, Angiotensin II could act as positive inotropic substance in the heart. This clinical hypothesis cannot be investigated at the moment because there is no appropiate animal model at the moment. A transgenic animal expressing heart chymase would, therefore, of great interest.

Another approach in transgenic research is the investigation of promoter-function and their tissue-specific expression, which, in future, allows to generate tissue-specific gene constructs to study genes of the cardiovascular system. Promoter sequences which are specific for expression of genes in the heart, kidney or vascular wall are currently investigated. Reporter genes like luciferase or chloramphenicol-acetyltransferase are genes to investigate promoter function and expression. The Myosin-light chain-2 promoter (Lee et al., 1992) has already been identified as a cardiac-specific promoter, which provides a powerful tool for future production of new animal models overexpressing genes only in the heart.

Future Aspects

One approach to study gene function is not only to inject new gene constructs into animals but also to generate so-called knockout animals. These animals typically are generated by homologous recombination of embryonic stem cells (Robertson et al., 1991). Some mouse models have already been established (Hosoda et al., 1995).

Another approach for inactivation and inhibition of gene expression is done by microinjection of antisense or ribozyme constructs. Up to now, there are only few reports of successful antisense targeting in vivo (Katsuki et al., 1988). A further possibility is to inject cytotoxic genes, like diphteria toxin A gene (Palmiter et al., 1987), which are under control of tissue-specific promoters. Activation of these promoters leads to cell death of the target cell. Expression of these genes during embryonic phase may cause death of the embryo. Therefore, promoters which can be pharmacologically activated are more preferable. The thymidine kinase gene from herpes simplex virus can be activated in the presence of certain nucleoside analogues such as gancyclovir. This activation induces cell death in vitro (Heyman et al., 1989).

In conclusion, the introduction of candidate genes into rats or mice provides a powerful tool to investigate cardiovascular diseases. These animals provide new possiblities of studying the etiology and pathophysiology of primary hypertension. The genetic basis of this disease will have a great deal of influence on diagnosis, prevention and therapy of this endemic disease.

Summary:
In the last years generation of genetically modified animals by transgenic techniques is an increasingly important tool in the field of hypertension research. Transgenic animals give the opportunity to analyse gene function and regulation in vivo.
In this review we want to give an overview of the applications of transgenesis to hypertension research, reviewing the most important transgenic lines which have been generated to date. Recent data about these transgenic animal lines is summarized. Finally we will give some information about new transgenic models and consider the future possibilities for hypertension research.

Resumé:
I løbet af de sidste år har transgene laboratorie dyr fået en stor betydning for forskningen af hypertension (forhøjet blodtryk). Ved brug af transgene laboratoriedyr er det muligt at undersøge funktionen af gener og hvordan generne reguleres in vivo.
Formålet med denne review artikel er at give et overblik over de områder af hypertension forskning hvor transgen teknologi er blevet anvendt og beskrive data for de vigtigste liner af transgene laboratoriedyr til hypertension forskning udviklet indtil nu. Til slut i denne artikel beskrives nye modeller og hvilke fremtidige aspekter den transgene teknologi har for udviklingen i hypertension forskningen.

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