Abstract. Hypertension is a significant cause of morbidity and mortality worldwide. It is defined as systolic and diastolic blood pressures (SBP/DBP) >140 and 90 mmHg, respectively. Individuals with an SBP between 120 and 139, or DBP between 80 and 89 mmHg, are said to exhibit pre-hypertension. Hypertension can have primary or secondary causes. Primary or essential hypertension is a multifactorial disease caused by interacting environmental and polygenic factors. Secondary causes are renovascular hypertension, renal disease, endocrine disorders and other medical conditions. The aim of the present review article was to examine the different animal models that have been generated for studying the molecular and physiological mechanisms underlying hypertension. Their advantages, disadvantages and limitations will be discussed.

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1. Introduction

Hypertension is one of the most important risk factors for the development of cardiovascular disease and is responsible for >50% of the 17 million deaths per year worldwide (1). It is a heterogeneous condition with a number of etiologies and multiple, interacting genetic and environmental factors (2). Its incidence varies with age, plasma renin activity and sodium sensitivity (3). The use of pre-clinical animal models has significantly increased our understanding of disease processes, as these permit the control of the different contributing factors. However, no single system is ideal, as there are species differences and other limitations of these model systems (4,5). Species, including mice, have been popular for the study of cardiometabolic disorders due to their amenability to genetic and pharmacological modification (6‑12). It is not the aim of the present review to provide an exhaustive list of the different models, but to discuss historically important model systems whose use has significantly advanced our understanding of hypertension.

2. Overview of different animal models

Animal models of hypertension can be categorised according to aetiology (13) (Fig. 1). Renal diseases, including renal arterial stenosis (RAS), are major causes of secondary hypertension. RAS has been modelled by the 2 kidneys-1 clip hypertension model (2K-1C), 1 kidney-1 clip hypertension model (1K-1C) and 2 kidney-2 clip hypertension model (2K-2C). Other systems have been devised to examine the pathophysiology of renal parenchymal hypertension, renal ischemia and perinephric fibrosis. A deoxycorticosterone acetate (DOCA)-induced model imitates the effects of mineralocorticoid- and glucocorticoid-induced hypertension. Pharmacological approaches using a nitric oxide synthase (NOS) inhibitor or by activating the renin-angiotensin-aldosterone-system (RAAS), or introduction of environmental stresses, including stress, cold temperature and diet, have also been performed to induce hypertension. Essential hypertension has been investigated using spontaneously hypertensive rats (SHRs), Dahl salt-sensitive and other rat strains (14). Other molecular models, transgenic strains, consomic and
congenic strains, combined with gene knockout techniques have been used to examine the mechanistic basis of essential hypertension.

3. Advantages and disadvantages of these model systems

Initial experiments for the investigation of hypertension were performed in dogs. Such experiments included the renovascular models developed by Goldblatt et al (15) in 1934. Subsequent models using rats, rabbits, sheep and cats were developed (16). Pigs have also been used, in particular, the Yucatan model for the study of DOCA-induced hypertension (17).

Of the different species, rat has been a popular model as a result of the availability of different inbred strains and characteristics, including the SHR, Dahl salt-sensitive rats, New Zealand and Milan strains (18). Numerous justifications for using rats to model hypertension exist. Firstly, its genome has been completely mapped, with a 99% sequence homology to humans (19). Secondly, the pathogenesis of hypertension in rats and humans are largely similar in terms of arterial pressure development from childbirth, response to environmental stressors, haemodynamic factors (including vascular resistance), mechanisms regulating arteriolar and venous constriction, neural modulation (including sympathetic nerve activity) and renal vascular dynamics (including perfusion parameters), as well as humoral influences by RAAS and NOS (20). The advantages are that they are low cost, with wide availability and easy to handle, maintain and breed. However, these models also have their limitations. Firstly, the identical genotype may not induce the same phenotype in all animals (21) due to contributions from numerous genes and the additional environmental influences (22,23). Secondly, the same gene mutations and deletion observed in rats may not induce to the identical phenotypic effects in humans (24). Larger animals have closer anatomical, physiological and haemodynamic properties to humans when compared with small animals, including rats and mice, making them particularly suitable for the study of flow characteristics (25,26). However, the major disadvantage is the high costs required for their maintenance. Additionally, the domestication of dogs has led to their decreasing use for research (27).

4. Renal models

Renovascular hypertension. The kidney-clip models mimicking renal arterial stenosis were first performed in dogs (15), which have been gradually replaced by smaller animals. In the 2K-IC model, one of the two renal arteries are constricted by a clip (28). Initially, decreased renal arterial pressure in the clipped kidney leads to increased plasma renin activity (PRA) with higher circulating levels of renin and aldosterone (29). This is followed by the return of the PRA to a near normal level, and finally by chronically elevated PRA (30). Patients with renovascular hypertension exhibit similar patterns of PRA (31). The underlying mechanism involves RAAS activation, increased renin production and subsequent angiotensin (Ang)-I release and conversion by Ang converting enzyme (ACE) to Ang-II. The net effects are further vasoconstriction and increased production of aldosterone level, which together lead to water and salt retention, and an increased blood pressure. In addition, the model also reveals increased sympathetic nerve activity that further drives renin production (32). The 2K-2C model, where both renal arteries are constricted, resemble bilateral renal arterial stenosis in humans and the mechanism involved is similar to the 2K-IC model, but with a more severe phenotype (33).

In the 1K-IC model, unilateral nephrectomy is performed with a constricting clip on the renal artery of remaining kidney (34). This resembles patients who suffer from RAS of the solitary kidney (35). Similar to the previous renal models, initial elevation of blood pressure is due to RAAS activation. However, because of the absence of a functional kidney, no compensatory rise in sodium and water excretion is observed. Consequently, more fluid is retained inside the body. In other words, this is more volume- rather than RAAS-dependent. This is consistent with the experimental findings that ACE inhibition was unable to prevent chronic hypertension in renal artery stenosis of a solitary kidney, however, was successful in doing so where a normal functioning kidney is present (36).

Renal parenchymal hypertension. Renal parenchymal hypertension is the commonest cause of secondary hypertension and is responsible for up to 5% of all cases (37). Subtotal nephrectomy ablation, in which up to 5/6 of the kidney is removed, has been performed to induce chronic renal disease (38). This model demonstrates glomerular, tubular and interstitial injury, loss of nephrons and the development of hypertension. It can be combined with the introduction of excess salt into the diet to increase the severity and speed of onset of this hypertension. The mechanism is dependent on the RAAS and the hypertension can be reduced by ACE inhibition. Renal ischemia has been produced by microembolisation, which led to the development of nephrosclerosis and hypertension (39). Perinephric fibrosis has been induced by wrapping the kidney in cellophane, mimicking fibrosis that occurs after kidney transplantation (40).

5. Pharmacological models

Mineralocorticoids or their synthetic derivatives, including DOCA, are used with sodium chloride in unilateral nephrectomised rats to induce hypertension (41,42). Renin is suppressed and fluid reabsorption is increased, thereby producing a low renin-volume overload model of hypertension (43). Using this model, key sodium-independent mechanisms for mediating hypertension, including upregulation of Ang-II receptors in the central nervous system (44), elevated vasopressin (45), increased oxidative stress (46) and endothelin (47), have been identified. Aside from elucidating the molecular mechanisms underlying renal hypertension, it provides a useful platform for investigating the natural history of disease, including any complications, such as glomerulosclerosis, proteinuria, impaired endothelium-dependent relaxation of the vasculature and cardiac hypertrophy can be investigated (42). In the DOCA-hypertensive Yucatan miniature swine model, excess dietary salt is not required for sustaining hypertension due to enhanced SNS activity at baseline, as evidenced by the increased plasma norepinephrine level (48). Glucocorticoids can also be used to induce hypertension (49). Although hypertension is produced via RAAS activation, this approach is
less effective than the DOCA-salt method. An alternative is chronic infusion of RAAS components, including Ang-II (50). Nitric oxide (NO), a potent vasodilator derived from the intact endothelium, is produced by NOS. This production is triggered by vasoactive messengers, including acetylcholine (51). A NO-deficient model was produced by chronic infusion of N-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor (52). A low dose produced a volume-dependent increase in blood pressure predominantly due to renal vasoconstriction and decreased glomerular filtration (53). A high dose led to both salt- and volume-independent hypertension since the mechanism is renal and systemic vasoconstriction (54).

6. Environmental models

Environmental stress, including separate or simultaneous introduction of flashing lights, loud noises and oscillating cages (55-57), or long-term exposure to high salt, fat or sugar in the diet, can be used to induce hypertension (58). Extremes of temperature, particularly coldness, also induces a hypertensive phenotype, as observed in rats exposed to 5°C for 3 weeks (59). In these animals, increases in plasma and urine catecholamines were observed (60). These findings are consistent with findings in humans, where those who work chronically in cold areas develop hypertension (61) and higher values of blood pressure recorded in winter compared with in the summer (62). Increased activity of the sympathetic nervous system and RAAS activation appear to be the common physiological mechanisms responsible for hypertension in the models described above (60,63,64).

7. Genetic models

Genetic factors are estimated to influence up to 50% of blood pressure variability in essential hypertension (65). The millennium genome project for hypertension was initiated in 2000 to identify genetic variants that predispose individuals to hypertension. This has involved a combination of techniques, including a gene linkage approach using single nucleotide polymorphisms, microsatellite markers and systematic candidate gene analysis (66). In parallel with this has been the development of genetic models using different animal species, which have provided insights into the physiological mechanisms of hypertension. These can be categorised into inbreeding, consomic, congenic and subcongenic strains (18), which will be considered in turn.

8. Inbreeding

The inbreeding method involves sibling mating of hypertensive rats over several generations to produce strains with genetic homogeneity when compared with the reference control group. 

Spontaneous hypertension models. SHR and stroke-prone SHR strains closely simulate essential hypertension (20,67). Both development impaired endothelium-dependent relaxation, cardiac hypertrophy and failure, as well as renal dysfunction, are involved (68). These represent normal renin, sodium-independent models of hypertension (69). SHRs were produced by breeding brother Wistar rats with their sisters and selecting the offspring with the highest blood pressures (70). In SHRs, increases in systolic blood pressures to 180-200 mmHg after 4 weeks of growth were observed, compared with the Wistar-Kyoto rats (WKY) that remain normotensive. It is worthwhile to note that WKY strains are not inbred, and therefore there is substantial genetic heterogeneity between these strains and between colonies within each strain (71). Consequently, no specific genetic components are associated with hypertension in the control WKY group.

SHRs have been used to determine the genes responsible for hypertension, to evaluate complications of target organs and the screening potential pharmacological agents for treatment. In stroke-prone SHRs, it was shown that dietary potassium supplementation decreases the risk of cerebrovascular accidents, even when blood pressure was not lowered (72). At least three genetic loci have been implicated in the early development of hypertension, with an additional gene identified on chromosome 10 contributing to its maintenance with aging (73). The New Zealand hypertensive rats are similar to Japanese SHRs in developing spontaneous hypertension (74), as do the Milan (20) and Lyon (75,76) strains.

Salt-sensitive hypertension models. Dahl salt-sensitive (DS) rat strains are prone to hypertension following administration
of a low-salt diet (0.4% NaCl), unlike Dahl salt-resistant (DR) rat strains, which remain normotensive (77). DS strain rats fed with a high-salt diet (8% NaCl) develop particularly severe hypertension (78). The reason is that the certain alleles at the genetic loci for ACE and guanylyl cyclase A, causing DS rats to have increased ACE and decreased atrial natriuretic factor (ANF, the ligand for guanylyl cyclase A) (79). The Sabra strain also demonstrates salt-sensitive hypertension (80).

**Other inbred models.** The Fawn hooded hypertensive rats develop hypertension due to glomerular sclerosis, and therefore serve as a model for renal parenchymal disease (81). Sprague-Dawley rats, obese Zucker, Wistar fatty rats have been used to assess the effects of diet-induced obesity on the development of hypertension (82).

9. Transgenic strains

Transgenic technology can be used to investigate the specific role of different genes in the regulation of blood pressure (83). Broadly, the approaches are generation of consomic and congenic strains, and gene knockout.

**Congenic and consomic strains.** A conogenic strain refers to one in which a defined chromosome segment is introduced to another by backcrossing with appropriate selection (84). In the case of a consomic strain, the entire chromosome is transferred (85). For example, the mutant renin gene from mouse was transferred to rats, producing elevated Ang-II levels and hypertension (86), which were prevented by ACE inhibition (87). Similarly, insertion of the human renin gene into mice also consistently demonstrated activation of genes involved in the RAAS (88,89).

**Gene knockout.** Gene targeting permits targeted disruption, including deletion, overexpression or subtle mutations, of a gene product. Conditional knockout with tissue- and time-dependent specificity is also possible, allowing investigation of the loss of a particular gene at specific time points or in particular organs. Gene knockout is often performed in mice because of the relative ease in introducing genetic mutations, and this has led to an increased understanding of different cardiovascular disorders with potential for translational application (90-99). Knockout of the angiotensinogen gene provided protection in delaying the development of hypertension (82).

**References**

12. Tse G, Yeo JM, Tse V, Kwan SK and Sun B: Gap junction inhibition by heptanol increases ventricular arrhythmogenicity by decreasing conduction velocity without affecting repolarization properties or ventricular refractoriness in Langendorff perfused mouse hearts. Mol Med Rep (In press).


111. Tse G: Both transmural dispersion of repolarization and transmural dispersion of refractoriness are poor predictors of arrhythmogenicity: A role for the index of Cardiac Electrophysiological Balance (QT/QRS)? J Geriatr Cardiol (In press).


