Abstract

With an average prevalence of 25% hypertension is one of the leading chronic diseases in westernized countries. Recent epidemiological data indicate a high proportion of patients with secondary hypertension due to primary aldosteronism that accounts for up to 8–12% of cases. Primary aldosteronism is caused by autonomous secretion of aldosterone by the adrenal cortex which results in hypertension with clinically, biochemically and therapeutically distinct features. With the exception of the small proportion of patients with familial hyperaldosteronism type I, the underlying genetic and molecular basis of this common disease is largely unknown. In this situation mouse models with targeted genetic modification can be utilized to define functional relevance of predefined candidate genes that are known or suspected to be involved in the regulation of aldosterone secretion. Moreover, animal models can aid in the identification of novel gene products that have not yet been identified to play a role in primary aldosteronism. This review will provide a brief overview on the animal models currently available for primary aldosteronism and describe in vivo screening strategies that are likely to provide insight in molecular and genetic mechanisms involved in autonomous aldosterone secretion.

© 2009 Elsevier Masson SAS. All rights reserved.

1. Primary aldosteronism - epidemiology and genetics

Primary aldosteronism (synonym: Conn’s syndrome) is characterized by an autonomous aldosterone excess with subsequent suppression of renin levels. Classical, hypokalaemic primary aldosteronism is a rare disease with prevalence rates of less than 1% within historical hypertensive series. Recent studies have suggested, however, that a normokalaemic variant of primary aldosteronism is much more common affecting 5 to 13% of the entire hypertensive population [1,2]. The majority of patients with primary aldosteronism have poorly controlled hypertension despite conventional multi-drug regimens. Several series have estimated the prevalence of primary aldosteronism in refractory hypertension (defined as treatment resistance despite three or more antihypertensive drugs including a diuretic) in the range of 17–31% [3,4]. Furthermore, in single center series hypokalaemic primary aldosteronism was associated with excess complications of hypertension, such as renal failure, left ventricular hypertrophy, cardiac fibrosis, and advanced atherosclerosis [5].

A small proportion of patients present with inheritable forms of primary aldosteronism with familial hyperaldosteronism: type I caused by a hybrid gene mutation formed by a cross-over of genetic material between the ACTH-responsive regulatory portion of the 11β-hydroxylase (CYP11B1) gene and the coding region of the aldosterone synthase (CYP11B2) gene and type II which is characterized by inheritance consistent with an autosomal dominant pattern of autonomous aldosterone hypersecretion which is not suppressible by dexamethasone. The precise genetic cause of type II familial hyperaldosteronism remains to be elucidated [6]. Although the genetic determinants of sporadic primary aldosteronism have also not been identified yet, recent data from the Framingham Heart Study [7] have demonstrated that the aldosterone to renin ratio is heritable and has modest linkage to chromosome 11p. A higher baseline log ratio was associated with increased risk of blood pressure progression and hypertension incidence indicating that apparently sporadic primary aldosteronism might be a heritable trait. To the contrary, a genome-wide linkage analysis of 2000 hypertensives of any origin and 3000 controls did not show any association to known or unknown loci [5].

2. Regulation of aldosterone secretion

The mineralocorticoid biosynthesis in the zona glomerulosa diverges from the glucocorticoid pathway at the level of
physiological conditions such as low-sodium balance, are not of the epithelial sodium channel has been replaced by the rat/H9251 deposit[16]. These findings together with experiments per-
diet lack elevation of blood pressure and overt cardiac collagen
tomized and aldosterone infused rats that were kept on a low-salt
[19]or potassium canrenoate [20]. Interestingly, uninephrec-
tionism including hypertension, left ventricular hypertrophy,
mineralocorticoid receptor antagonists, such as spironolactone
corticosterone acetate, and antagonized by the administration of
mimicked by the administration of the mineralocorticoid deoxy-
corticosterone to corticosterone and subsequently to aldosterone [8]. The primary secretagogues that stimulate aldosterone biosynthesis are angiotensin II (AngII), ACTH and potassium. AngII modifies several aspects of adrenal cell metabolism and physiology including steroid secretion and zona glomerulosa cell proliferation. ACTH has only transient stimulatory effects on aldosterone secretion and is not required for normal aldosterone output as evident in patients with secondary adrenal insufficiency [9]. However, upregulation of the ACTH receptor in aldosterone secreting adenomas has been suggested to be one of the mechanisms of ACTH responsiveness in these tumors [10]. Potassium ions directly stimulate aldos-
stoner secretion, independent of any effect on the circulating renin-angiotensin system. The glomerulosa cell is characterized by its distinct potassium sensitivity with an acute increase in serum potassium of 0.1 mmol/l resulting in up to 25% increase in serum aldosterone secretion [11]. Thus, potassium is involved in a negative short feedback loop with aldosterone: increased potassium levels stimulate aldosterone secretion which in turn increases renal potassium loss and vice versa.

While ACTH acts primarily via increase of intracellular cAMP, AngII and potassium both elevate cytoplasmatic calcium levels through activation of phospholipase C which is followed by inositol-trisphosphate generation and release of Ca2+ from intracellular stores [12]. This increase in intracellular Ca2+ results in activation of calmodulin and CaM kinases [13] that in turn lead to activation of transcription factors including CREB and NGFI-B [14] and finally promoter activation of steroidogenic enzymes including aldosterone synthase [15].

3. Pharmacological models of primary aldosteronism

Experimental studies performed in rats that were uninephrec-
tomized and additionally exposed to a high salt diet (1.0% NaCl drinking water) and chronically infused with aldosterone (0.75 μg/h for 8 weeks) have been utilized in the past as a model of high aldosterone states [16]. These animals have been demonstrated to develop a phenotype similarly to primary aldosteronism including hypertension, left ventricular hypertrophy, and cardiac fibrosis [17,18]. These phenotypic endpoints can be mimicked by the administration of the mineralocorticoid deoxy-
corticosterone acetate, and antagonized by the administration of mineralocorticoid receptor antagonists, such as spironolactone [19] or potassium canrenoate [20]. Interestingly, uninephrec-
tomized and aldosterone infused rats that were kept on a low-salt diet lack elevation of blood pressure and overt cardiac collagen deposit [16]. These findings together with experiments performed on transgenic mice in which the endogenous α-subunit of the epithelial sodium channel has been replaced by the rat α-
ENaC subunit under the control of the cytomegalovirus promoter [21] suggested that high aldosterone levels, when appropriate to physiological conditions such as low-sodium balance, are not by themselves detrimental to the heart or the kidney. In contrast, these experiments indicate that the co-occurrence of excessive salt loading with inappropriately high aldosterone level is the cause of renal damage and heart remodeling.

4. Genetically modified animals with primary aldosteronism

Despite the fact that sequencing approaches have revealed extensive information on the number of potential genes and their distribution over the genome, most of the functional properties of biological molecules are still unpredictable from pure sequence analysis. Likewise, although in vitro experiments can provide detailed insight into mechanisms of action of a given gene product, for further functional analyses, mouse models are intensively utilized as an experimental system due to the similarity to humans with respect to genome organization, developmental and biochemical pathways and physiology. As for primary aldosteronism a number of candidate genes have been pro-
posed to be potentially involved in aldosterone autonomy: in line with the functional significance of angiotensin II and potassium as the main physiological regulators of aldosterone secretion, mice with genetic modification of the underlying molecular pathways involved in angiotensin II and potassium dependent aldosterone secretion have been developed and characterized for their endocrine and cardiovascular phenotype.

Recently, a mouse model has been described in which the wild type angiotensin II receptor type 1A had been replaced with a gain-of-function mutant of this receptor, associating a consti-
tutively activating mutation with a C-terminal deletion, which impairs receptor internalization and desensitization [22]. As expected, in this knock-in mouse model constitutive activation of the angiotensin II receptor was associated with hypertension and development of cardiac, vascular, and renal fibrosis. Of note, although very low plasma renin levels were evident that resulted in a clear elevation of aldosterone to renin ratio, aldosterone secretion itself was unaltered in comparison to wild type con-
trols [22]. Thus, this endocrine profile differs from laboratory findings usually present in patients with primary aldosteronism.

Sensing of potassium concentration by the glomerulosa cell is dependent on the presence of specific potassium channels which are involved in membrane depolarization and, thus, stimu-
lation of aldosterone secretion. As such, alteration in expression and/or function of members of these potassium channels have been suggested to contribute to aldosterone autonomy. Interest-
ingly, animals with targeted deletion of the large-conductance, voltage- and Ca2+-activated K+ (BK) channel have been dem-
strated to exhibit a significant blood pressure elevation which is accompanied by hyperaldosteronism and decreased serum potassium levels [23]. While these findings are in line with autonomous aldosterone secretion as the driving force of the described phenotypic abnormalities, increase in blood pressure could also be attributed to BK channel dependent vascular abnor-
malities which result in increased myogenic vessel tone [23]. As BK channels are not constitutively open at rest, it has been proposed that background conductance necessary to confer the unique extracellular potassium sensitivity of glomerulosa cells could not solely depend on the function of these channels [24].
Furthermore, electrophysiological recordings of glomerulosa cells have emphasized the importance for leak-type potassium channels of the 2P domain family [25]. In accordance with this notion, two independent groups recently described a mouse model with autonomous aldosterone secretion upon targeted deletion of 2P domain family potassium channels TASK1 [26] and TASK1 and TASK3 [24], respectively. Phenotypic characterization of these animals provided evidence that deletion of TASK1 results in impaired mineralocorticoid homeostasis accompanied by salt retention, arterial hypertension associated with low plasma renin activity. These endocrine abnormalities were associated with a (gender specific) zonation defect of the adrenal cortex with aberrant aldosterone synthase expression in the zona fasciculata [26]. Thus, these studies clearly indicate that genes that are required for normal aldosterone regulation – for example by sensing potassium levels within the zona glomerulosa cell – can be involved in the development of primary aldosteronism. However, as the aldosterone secretion in affected mice was remediable by glucocorticoid treatment [26] which is not the case in the vast majority of patients with primary aldosteronism it is evident that there are very likely other genetic contributors for aldosterone autonomy which have not been identified to date.

5. In vivo models to identify novel genes involved in aldosterone regulation

In an attempt to explore global potassium dependent transcriptional changes in the adrenal gland we have recently established a well-defined long-term substitution experiment: wild type male mice were kept on a low (7 g potassium per kg chow), normal (9 g potassium per kg chow) and high potassium diet (11 mg potassium per kg chow) for 5 weeks, after which animals were euthanized, trunk blood collected and adrenals removed and micro-dissected. As expected, hormone analysis revealed a significant increase in plasma aldosterone levels under high potassium diet and a concomitant increase in adrenal aldosterone synthase expression levels and slight increase in the thickness of the zona glomerulosa. This paradigm of zona glomerulosa stimulation was utilized to define transcriptional changes in the adrenals of the experimental groups. Specifically, gene array analysis defined adrenal genes that displayed opposite changes in expression levels in dependence of potassium intake. Whether these genes are the cause or consequence of zona glomerulosa stimulation and, thus, of importance for autonomous aldosterone secretion in primary aldosteronism has to be defined in further expression studies: autonomous aldosterone secretion in Conn’s adenomas is accompanied by low renin levels and (a tendency to) hypokalaemia. Thus, expression analysis of the pre-defined candidate genes will provide evidence whether regulation of the gene in a potassium dependent manner is still intact (down-regulated) or possibly part of the autonomous dysregulation (up-regulated). Together with functional in vitro experiments these findings could help to identify candidate genes involved in autonomous aldosterone secretion.

In addition, to mouse models with targeted genetic modification as described above different approaches have been used to create informative mouse models, known as forward (phenotype-driven) genetics. N-ethylnitrosourea (ENU) is cur-

Fig. 1. Schematic presentation of experimental setup of a mutagenesis screen for hyperaldosteronism and potential application of resulting animal models.
rently the most powerful mutagen for the production of mutants in mice [27]. The mutations recovered after ENU-mutagenesis are mainly point mutations, i.e. A-T base pair substitutions and/or small intragenic lesions [28]. Although gain-of-function as well as complete loss of function mutants can also be expected many of the mutants produced by ENU will reveal a partial loss-of-function of the affected gene product [29]. ENU displays mutagenic action on pre-meiotic spermatogonial stem cells [30] which allows the production of a large number of F1 founder mice from a single treated male animal.

For further genetic examination of primary aldosteronism, we recently initiated a systematic, genome-wide production and analysis of mouse mutants with high aldosterone levels. In these ongoing studies which utilize a newly designed aldosterone assay for screening purposes [31] a number of mouse lines with primary aldosteronism have been established that await further phenotypical and molecular characterization (Fig. 1). Although preliminary in its experimental design due to the low animal number our data provide solid evidence for the usefulness of the generated animals as models of primary aldosteronism. Although detailed molecular analysis of adrenals from affected animals might help to pinpoint a pathway with potential impact in dysregulated aldosterone secretion in the individual mouse line the specific underlying genetic cause is unlikely to be determined solely on the basis of phenotypic techniques. Thus, a genetic approach will be required for the identification of the mutation induced by the initial chemical mutagenesis.

Taken together, animal models of primary aldosteronism can be utilized to define specific aspects of the endocrine and metabolic disorder responsible for the distinct cardiovascular phenotype such as inappropriately high aldosterone secretion with respect to salt intake. In addition, genetically modified mouse models can provide insight into pathophysiological significance of specific molecular mechanisms for autonomous aldosterone secretion. Furthermore, in vivo screening approaches that base on physiological stimulation experiments or on chemically induced mutagenesis could aid in the definition of novel candidate genes with potential relevance in patients with sporadic primary aldosteronism. Finally, definition of cardiovascular and metabolic endpoints in well-characterized mouse models with hyperaldosteronism as outlined above will be the basis for future pharmacologic intervention studies.

References


