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Review

The mineralocorticoid signaling pathway throughout development: Expression, regulation and pathophysiological implications

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ABSTRACT

The mineralocorticoid signaling pathway has gained interest over the past few years, considering not only its implication in numerous pathologies but also its emerging role in physiological processes during kidney, brain, heart and lung development. This review aims at describing the setting and regulation of aldosterone biosynthesis and the expression of the mineralocorticoid receptor (MR), a nuclear receptor mediating aldosterone action in target tissues, during the perinatal period. Specificities concerning MR expression and regulation during the development of several major organs are highlighted. We provide evidence that MR expression is tightly controlled in a tissue-specific manner during development, which could have major pathophysiological implications in the neonatal period.

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1. Introduction

1.1. Aldosterone

Aldosterone, discovered and purified in 1953 by Sylvia and James F. Tait [1,2], is a cholesterol derived steroid hormone and the main hormone of the mineralocorticoid signaling pathway. Its synthesis in the adrenal *zona glomerulosa* [3] is tightly regulated by the expression of the aldosterone-synthase or *CYP11B2* gene, specific to aldosterone biosynthesis [4], localized on chromosome 15 in mice [5] and on 8q22 in human [6]. This gene, conserved among species, presents 95% homology with the *CYP11B1* gene, located 40 kb upstream *CYP11B2*, which encodes the enzyme 11-beta hydroxylase catalyzing the last step of cortisol (in humans) or corticosterone (in rodents) biosynthesis [6].

Abbreviations: MR, mineralocorticoid receptor; ENaC, epithelial sodium channel; GW, gestational weeks; GR, glucocorticoid receptor; 11βHSD2, 11 beta-hydroxysteroid dehydrogenase type 2; CCD, cortical collecting duct; TonEBP, tonicity-responsive enhancer binding protein; ES cells, embryonic stem cells; Tis11b, TPA-inducible-sequence 11b.

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Most mineralocorticoid hormones are synthesized in the adrenal gland. However, recent studies have raised the possibility of a local aldosterone biosynthesis [4]. Indeed, the *CYP11B2* enzyme has been found in the central nervous system (CNS) [7,8] and the cardiovascular system [9,10], particularly in the fetus [10]. The physiologic relevance of these local productions and their autocrine or paracrine actions are yet to be determined but could play a prominent role during brain or heart development.

Actually, the principal role for aldosterone concerns sodium homeostasis, which has been well documented in both human and animals: it contributes to sodium reabsorption and potassium excretion in the distal nephron thus participating to the control of plasma volume and blood pressure. This is particularly evident in different pathologies associating aldosterone excess and hypertension [11–15]. On the contrary, the absence of aldosterone biosynthesis, resulting from homozygous mutations in the *CYP11B2* gene, induces urinary sodium wasting which leads to dehydration and vital risk in newborns and requires sodium supplementation [16–18].

Aldosterone appears therefore as the main hormone controlling sodium homeostasis in mammals, with a pivotal role during the first weeks of life. The main biological effects of aldosterone at the cellular level are mediated through genomic and non-genomic pathways. The classical, well described genomic pathway, implies its binding to the specific mineralocorticoid receptor (MR), a transcription factor, which undergoes hormone-dependent nuclear

translocation and binds specific hormone responsive elements leading to transactivation of various target genes [19]. The non-genomic pathway, less characterized to date, involves numerous signaling pathways that are largely dependent on the tissue and cellular context [20]. It remains to be established whether a specific receptor, either associated to the cell membrane – or the classical cytosolic MR, could mediate such non-genomic effects [21–24].

1.2. The mineralocorticoid receptor

The *NR3C2* gene encoding the human MR (hMR) was cloned in 1987 [25]. It is localized on chromosome 4 at the q31.1–q31.2 locus and spans approximately over 450 kb [26,27]. It is composed of ten exons and eight introns [28]. The first two exons 1 α and 1 β encode the 5' untranslated region of the transcripts and are alternatively spliced into exon 2. This alternative transcription is under the control of two distinct promoters, P1 and P2, whose activities have been largely characterized in all MR target tissues [29]. The final 984 aa protein is composed of the classical three domains of steroid receptors: the N-terminal domain, the DNA binding domain and the ligand binding domain [30]. The mouse *MR* gene has a genomic structure similar to the rat *MR* gene, with at least 3 untranslated exons in the 5' region (unpublished results).

Aldosterone and glucocorticoids (cortisol in humans or corticosterone in rodents) are all able to bind MR with the same affinity. Mineralocorticoid selectivity is therefore essential to prevent permanent MR occupancy by cortisol since its plasma levels are 100- to 1000-fold higher than those of aldosterone. This selectivity is ensured in epithelial target tissues by the 11 beta-hydroxysteroid dehydrogenase type 2 (11 β HSD2) enzyme [31,32]. This enzymatic activity specifically converts cortisol (or corticosterone) into cortisone (or 11-dehydrocorticosterone in rodents), in a NAD (Nicotinamide Adenine Dinucleotide)-dependent manner. Interestingly, analogously to MR [33] and aldosterone [34,35], this enzyme appears pivotal in the postnatal period but is dispensable during fetal development since neither its invalidation in the mouse [36] nor homozygous inactivating mutations in humans [37] affect normal embryonic and fetal development, even though 11 β HSD2 is expressed early in gestation [38–40].

For decades, MR expression was considered to be mostly restricted to polarized tight epithelia, where MR, coexpressed with 11 β HSD2, is involved in aldosterone-dependent transepithelial sodium transport [41]. These MR expression sites, initially identified by binding assays or immunohistochemistry, included distal convoluted tubules and cortical collecting ducts of the kidney [42,43], distal colon [44], airway epithelia of the lung [45,46], and the salivary [47] and sweat glands [48]. Since then, new MR expression sites have been uncovered: the eyes [49–53], the placenta [54], the uterus and testis [55] and keratinocytes [56,57]. More importantly, MR expression has also been detected in unexpected non-epithelial tissues such as the heart [58,59], large vessels [60], the dentate gyrus and CA1, 2 and 3 nuclei of the hippocampus, and the hypothalamus [61–63]. MR expression has also been reported in leukocytes, macrophages [64,65] and pancreas [66,67]. Finally, our group and others have identified the brown and white adipose tissues as new target sites for MR action [68–73]. As a result, today, MR obviously interests physiologists, endocrinologists, cardiologists and nephrologists, but also pediatricians concerned by the hormonal regulation of the “Milieu Intérieur” particularly in newborns.

2. Mineralocorticoid signaling and development

The integrity of the mineralocorticoid signaling pathway is pivotal during the perinatal period, especially for adaptation to the

change from intra-uterine to extra-uterine life. In this review, we will mainly focus on the developmental specificities of mineralocorticoid signaling expression and regulation in several major target tissues that have been discovered in the past few years and which uncover a new exciting role for mineralocorticoid action.

2.1. Kidney

The kidney is obviously one of the major epithelial tissues submitted to drastic changes in the perinatal period. The critical role of the renal mineralocorticoid signaling pathway in neonatal adaptation to a new environment has been emphasized by numerous animal models and human pathophysiological situations. Indeed, MR-null mice die around day 10 after birth from major salt loss [33]. Newborns can be rescued by NaCl injections subsequently replaced by oral supplementation after weaning [74]. Interestingly, newborn mice exhibit no gross developmental defects [75], but develop life-threatening dehydration in the early postnatal life concomitant with the end of renal maturation (postnatal day 8 in mice) [76]. Similarly, embryonic development of *CYP11B2* knockout mice is not altered, but deadly sodium wasting and dehydration are observed in one third of newborn pups after the first week of life [34].

In humans, inactivating mutations of the *MR* gene result in a very similar phenotype. Autosomal dominant pseudohypoaldosteronism type I (PHA1) is characterized by renal resistance to aldosterone action. Patients present during the first weeks of life with dehydration, failure to thrive, hyperkalemia, hyponatremia, and metabolic acidosis despite extremely high aldosterone and renin plasma levels. Sodium supplementation is required for survival of these children, which can be subsequently replaced by a high sodium diet [19,77,78]. Interestingly, except the exceptional case of severe recessive PHA1 caused by two heterozygous MR mutations [79], no homozygous MR mutation has been described to date, suggesting a possible lethal phenotype incompatible with fetal development in humans, at variance with mice [33]. Similarly, homozygous mutations in the *CYP11B2* gene resulting in an absence of aldosterone biosynthesis induce urinary sodium wasting that leads to dehydration and vital risk in newborns and requires sodium supplementation [16–18]. Interestingly, these enzymatic defects do not alter fetal development, even though *CYP11B2* expression, at the mRNA and protein levels, begins as early as 13 gestational weeks (GW) in human fetal adrenals [80,81], in parallel with the onset of fetal aldosterone secretion [82]. However, an abnormal aldosterone synthesis during the first weeks of life is deleterious, demonstrating that the mineralocorticoid signaling pathway is instrumental for normal development in the postnatal period, and particularly after the first week of life.

Along this line, our group has recently demonstrated the existence of a transient physiological renal resistance to aldosterone in the early neonatal period [83]. Indeed, we could detect a significant difference between aldosterone and renin plasma levels of 50 pregnant women in labor and umbilical cord blood hormonal levels of their respective full-term and healthy newborn children. The very high levels of aldosterone and renin measured in newborn cord blood samples contrasted with signs of functional hypoaldosteronism at birth, including hyperkalemia, hyponatremia, and urinary salt waste all features of a partial renal insensitivity to aldosterone (Fig. 1A). This hormonal resistance could account for the weight loss observed during the first days of life and might be an adaptive phenomenon of the passage from the *in utero* aquatic life to terrestrial life. To investigate the possible underlying molecular mechanisms, we formulated the hypothesis that this aldosterone unresponsiveness could be related to a variation of MR expression during renal ontogenesis and chose to explore MR expression throughout fetal development. MR transcript and

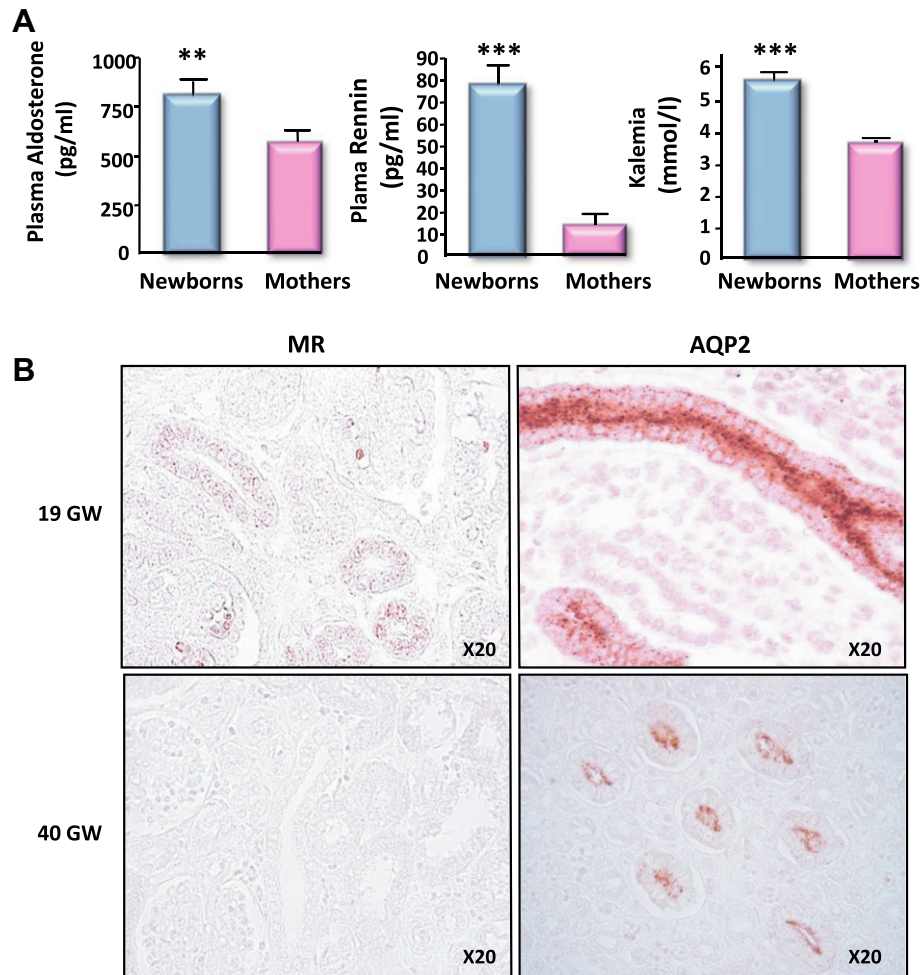


Fig. 1. A. Comparison of aldosterone, renin and potassium concentrations between umbilical cord blood of 48 healthy full-term newborns and maternal plasma. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. B. Immunodetection of mineralocorticoid receptor (MR) and aquaporin 2 (AQP2) proteins in human fetal kidneys. MR protein is detected in the nucleus of cortical collecting duct cells at 19 GW [140] but not at 40 GW, whereas AQP2 is observed at the apical membrane of the same cells at both developmental stages. Original magnification: $\times 20$. MR monoclonal antibody [140] was used at dilution 1:120, AQP2 monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used at dilution 1:1000.

protein levels were analyzed at various developmental stages in both murine and human renal samples by quantitative RT-PCR, immunohistochemistry, and immunoblotting. We demonstrated that MR is transiently expressed in human convoluted distal tubule cells between 15 and 24 GW [40], and between 16 and 18 days *post-coitum* in the mouse, which corroborated previous studies during early gestation in humans [39,84] and mice [38]. In the prenatal period and especially at birth, renal MR expression is down-regulated, with MR mRNA and protein levels falling below the detectable threshold. This cyclic MR expression profile correlates with that of α ENaC, a major MR target gene and 11 β HSD2, which confers mineralocorticoid selectivity, at variance with other genes expressed in the CCD, such as aquaporin 2 (Fig. 1B), thereby identifying a temporal cyclic expression pattern specific to the mineralocorticoid signaling pathway conserved between species [40].

In a recent paper, our group has also emphasized the specific and crucial role played by the mineralocorticoid signaling pathway in the postnatal period. Using both the aldosterone-synthase knockout mouse model, and organotypic cultures of newborn mouse kidneys, we demonstrated that whereas aldosterone has no impact on MR neonatal downregulation, it is required for MR and α ENaC optimal upregulation in the postnatal period, and is therefore indispensable for sodium homeostasis [85]. Even though the

glucocorticoid signaling pathway is readily expressed, functional at birth [40] and has been shown to regulate α ENaC expression in other models [86], it cannot replace the mineralocorticoid pathway in the neonatal period *in vivo* [85].

This contrast between high aldosterone levels at birth and the low renal MR expression is very intriguing. Whether it corresponds to a protective mechanism of the kidney against high aldosterone levels required elsewhere for activation of genomic or non-genomic pathways [20] or whether the absence of an active renal mineralocorticoid pathway is necessary in the first days of life for adaptation to a non-aquatic environment, remains to be elucidated. Very interestingly, while 11 β HSD2 activity is still very high in the placenta in the prenatal period in order to protect the fetus from excessive maternal glucocorticoids, the renal 11 β HSD2 activity is extremely low at birth [87]. Thus, the kidney displays a very specific window in the perinatal period, where the mineralocorticoid signaling pathway is virtually absent and the glucocorticoid signaling pathway, with a functional GR observed in the nuclei of the cortical collecting duct cells [40] and detectable levels of circulating cortisol [87], can be activated. This specific temporal window could be of major importance for renal maturation and developmental programming, as suggested by several authors [88,89].

In sum, it appears that a downregulation of MR expression is mandatory at birth both in mouse and human, while a certain and appropriate level of MR expression is required for normal development in the postnatal period [90].

Another intriguing question concerns the possible regulatory mechanisms implicated in this transient renal MR upregulation in the fetus and its successive downregulation at birth. During pregnancy, the fetus develops in an aquatic environment, and is submitted to physiologic osmotic variations of the amniotic fluid. Indeed, the composition of the amniotic fluid changes throughout development, especially regarding sodium concentration. We recently demonstrated the involvement of osmotic stress in the regulation of MR expression. The use of the highly differentiated renal KC3AC1 cell line, which forms domes at confluence, maintains endogenous MR expression and responds to physiologic concentrations of aldosterone [91] enabled us to demonstrate that extreme fluctuations of the extracellular fluid composition modulate MR expression *via* post-transcriptional mechanisms. Indeed, hypertonic stress inhibits MR gene transcription and/or accelerates MR transcript decay. Renal cells resist hypertonic stress by producing intracellular osmolytes (betaine, myoinositol, sorbitol) to reduce intracellular ionic strength. This relies on the tonicity-responsive enhancer binding protein (TonEBP or NFAT5/OREBP), a transcription factor belonging to the Rel family [92,93] that is recruited onto tonicity responsive elements (TonE), of tonicity-regulated target genes. Hypertonicity upregulates TonEBP activity through combined enhancement of TonEBP transcription, induction of TonEBP phosphorylation and increase of TonEBP nuclear localization. Of interest, we recently showed that the gene *Tis11b* (TPA-inducible-sequence 11b, also named ZFP36L1 or BRF1) is a direct target of TonEBP. *Tis11b* encodes a protein belonging to a family of zinc-finger RNA binding proteins involved in destabilization and degradation of short-lived mRNA [94,95]. *In silico* analysis of the human *Tis11b* promoter revealed the presence of 4

highly conserved TonE, consistent with a TonEBP-mediated *Tis11b* transcription in renal cells. Upon hypertonic stress, TonEBP expression in KC3AC1 cells increases in a time-dependent manner, with a parallel increase of *Tis11b* transcript and protein levels [91]. *Tis11b* interacts with AU-rich elements (AURE) located in the 3'-untranslated region (3'-UTR) of several short-lived mRNAs, thus targeting host AURE-harboring mRNA towards rapid degradation. We have identified seven AURE consisting of pentamers of AUUUA or nonamers of UUAUUUA(U/A)(U/A), downstream of the stop codon TGA in exon 9 of human *hMR* gene, which are highly conserved between species. This suggests that these regulatory motifs could represent direct *Tis11b* binding targets. Interestingly, a significant negative correlation between MR and *Tis11b* expression was observed, providing additional support for a functional relationship between high expression of *Tis11b* and a low MR expression in KC3AC1 cells submitted to hypertonic stress and *vice versa* (unpublished data).

Taken together, we provide strong evidence that TonEBP and *Tis11b* are central players in the control of renal MR abundance under hypertonic stress. A proposed model for this post-transcriptional control of renal MR expression by *Tis11b* RNA binding protein is presented in Fig. 2. Under hypertonic stress, TonEBP expression is increased, followed by nuclear translocation of the phosphorylated transcription factor that binds to the TonE responsive elements of *Tis11b* promoter, hence stimulating *Tis11b* gene transcription. This leads to an accumulation of *Tis11b* protein that, *via* binding to the AURE of the 3'-UTR of MR mRNA, accelerates its degradation and subsequently reduces MR abundance in renal cells.

From a phylogenic point of view, this phenomenon is reminiscent of variations of MR expression observed in teleost fish during their acclimation from fresh to seawater and *vice versa*. Indeed, MR expression is downregulated after transfer to salt water during smoltification [96], supporting the notion that osmoregulatory

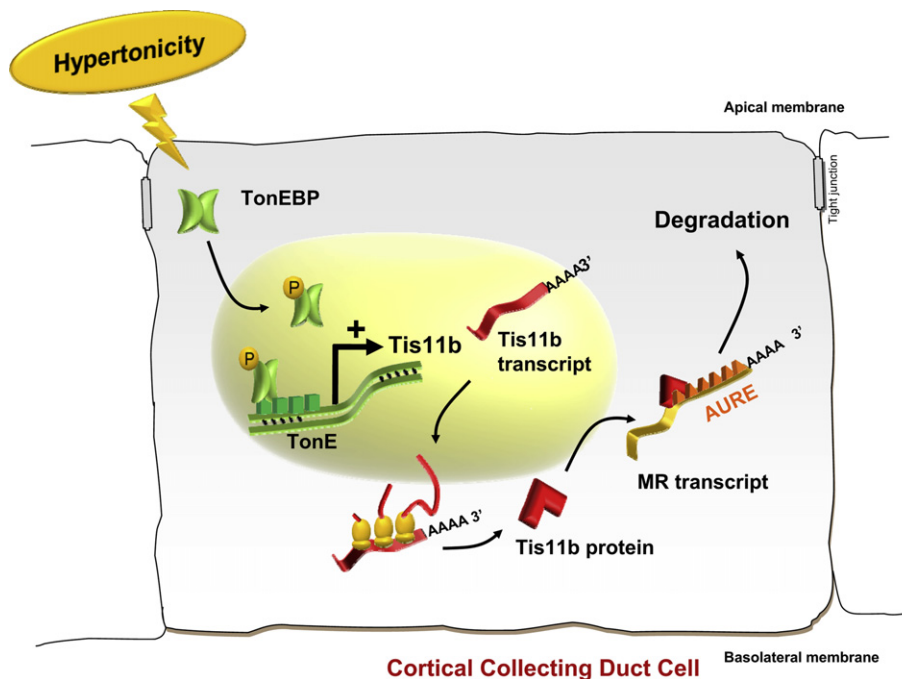


Fig. 2. Proposed model for post-transcriptional control of renal MR expression by *Tis11b* under hypertonic stress. Under hypertonic stress, TonEBP expression is increased, followed by nuclear translocation of the phosphorylated transcription factor that binds to the TonE responsive elements of the *Tis11b* promoter, hence stimulating *Tis11b* gene transcription. This leads to an accumulation of *Tis11b* protein that, *via* binding to the AURE of the 3'-UTR of MR mRNA, accelerates its degradation and subsequently reduces MR abundance in the CCD cells. AURE, AU-rich element; MR, mineralocorticoid receptor; TonE, tonicity response element; TonEBP, tonicity-responsive enhancer binding protein; *Tis11b*, TPA-inducible-sequence 11b.

mechanisms tightly control MR signaling. Similar mechanisms could therefore account for variations of renal MR expression during fetal development and could represent interesting new areas of research to explore.

2.2. Brain

Among other MR-expressing tissues, the central nervous system (CNS) represents an interesting target tissue with regards to development. In the adult CNS, MR is physiologically occupied and activated by glucocorticoid hormones rather than by aldosterone. Aldosterone-selective MR signaling is, however, activated in hypothalamic sites and is involved in the regulation of salt-appetite [97]. MR is highly expressed in the limbic structures, particularly in the hippocampus, where it is involved in the regulation of the hypothalamic–pituitary–adrenocortical axis activity under basal and stress conditions [98–100]. MR activity is also associated, at low glucocorticoid levels, with cell excitability and long-term potentiation, pivotal for learning ability and memorization [101]. Transgenic animal models have provided further insights into the CNS function of MR. Indeed, targeted MR overexpression in the forebrain leads to a reduced anxiety-like behavior coupled with a diminished GR expression and an increased serotonin 5HT-1a receptor expression [102]. It also enhances memory and reduces neuronal decay after cerebral ischemia [103]. On the contrary, conditional MR knockout in the forebrain results in impaired spatial learning ability and emergence of behavioral stereotypes [104].

Since the most critical period for brain development occurs during the two postnatal years, expression and regulation of MR expression in the CNS during the perinatal period appears to be of major importance. MR ontogeny is best documented in the hippocampus. Various mouse studies have demonstrated that MR hippocampal expression appears between E14 and E15.5 [38,105,106]. Thereafter, MR mRNA levels decrease until postnatal day 10 (D10), then increase slightly between D10 and D20, before its expression stabilizes [106]. Unpublished data from our group corroborate this cyclic MR expression in mouse hippocampus, with a specific decrease around birth (Fig. 3, left panel). This antenatal MR expression peak could emphasize a role of this receptor during prenatal hippocampal or HPA-axis development [107]. It is interesting to note that expression of GR transcripts is detected only from D5 onward [106]. These differential expression patterns have already been reported in rat brain [108,109]. In contrast, in the human hippocampus, MR and GR are both expressed at 24 GW, but no variation is observed until the 34th GW [106]. However, since it is extremely difficult to obtain fetal brain samples before 24th GW and after 34th GW, whether human MR expression follows a similar temporal pattern than that in mice remains to be established. Very few studies have examined prenatal pituitary MR expression. Porter et al., have demonstrated that MR transcript levels decrease during embryonic development in the chicken [110]. On the contrary, MR postnatal pituitary expression has been more largely analyzed in rats [111,112], mice [113], and primates [114]. In all these species, MR expression increases after birth and then stabilizes up to adulthood. In conclusion, MR hippocampal and pituitary expressions are conserved between species, with a specific temporal expression window around birth, similar to that observed in the kidney. MR expression is also tightly regulated by the existence of two spliced variants, as described earlier [115,116]. These isoforms (α and β) are differentially regulated during brain development [117]. While isoform α and total expression of MR transcripts are stable during development, isoform β is essentially expressed during the first two weeks of life. Finally, since MR exerts many important functions in the CNS, we have investigated its

expression during neuronal differentiation, using an embryonic stem (ES) cell model recapitulating the main steps of neural embryonic development. We observed an increasing MR expression throughout neuronal differentiation and established that both P1 and P2 promoter activities tightly correlate with MR expression during neuronal differentiation [118]. Collectively, these data indicate that alternative promoters determine the spatio-temporal expression of the MR gene during CNS development. This does not exclude that post-transcriptional and post-translational mechanisms might also be implicated in the control of MR expression and function in the brain thereby affecting optimal adaptation later in adulthood. We also provided evidence that MR acts as an important neuroprotective factor [119] that could play a significant role during neurogenesis and exert a protective effect against oxidative-stress neuron apoptosis [120]. If and how MR may affect neuronal growth, function and survival during development remains to be further evaluated.

Of interest, it has recently been demonstrated that, at variance with adults where no 11 β HSD2 expression and activity can be detected in the brain, the fetal brain expresses high levels of 11 β HSD2, in rat, mice and humans [38,121]. Thus, it can be speculated that during this specific temporal window, high 11 β HSD2 may protect the developing brain from excessive activation of MR/GR by glucocorticoids, and could favor a specific aldosterone-MR signaling in the fetus. In the neonatal period, 11 β HSD2 gene expression becomes more restricted, which could allow increasing glucocorticoid activation of GR and/or MR (regulated by sequential expression of these two receptors) permitting key glucocorticoid-dependent neuronal and glial maturational events to occur.

2.3. Heart

MR ontogeny during heart development has only been scarcely studied to date. An extensive study using *in situ* hybridization during mouse development has reported an onset of MR mRNA expression in the heart starting from E13.5. Its expression greatly decreases by E18.5 but reappears postnatally, with a similar temporal expression profile than in the kidney and the CNS [38]. More recently, the expression of MR and GR was examined in the heart of sheep fetuses [122]. A significant decrease of MR and GR expression was detected from day 80 of gestation to the postnatal stage in the left but not in the right ventricle. Moreover, it was recently shown that *in utero* cortisol treatment increased the heart weight/body weight ratio in lambs [123,124], such an effect being mediated by GR and/or MR. This may indicate a variable or sequential activation of mineralocorticoid and glucocorticoid signaling pathways along with cardiac development that could account for the final imbalance between the muscular thickness of the right and left ventricles. Indeed, several studies using genetically modified mice have brought new insights into the function of MR during the development of the cardiovascular system. Overexpression of the human MR gene during murine development induces cardiac hypertrophy, arrhythmia and tachycardia [125]. Moreover, conditional overexpression of the MR gene in cardiomyocytes using the doxycycline tetO system [126] results in life threatening arrhythmia when the transgene is activated *in utero* and can be prevented by maternal doxycycline treatment (thus blocking transgene expression in the fetuses) or by spironolactone treatment. It would be of particular interest to use these conditional MR knockout mouse models to gain further insight into the specific function of MR during cardiomyocyte differentiation and global heart development.

Recently, our group has generated recombinant ES cell lines, derived both from *hMR*-overexpressing transgenic blastocysts and

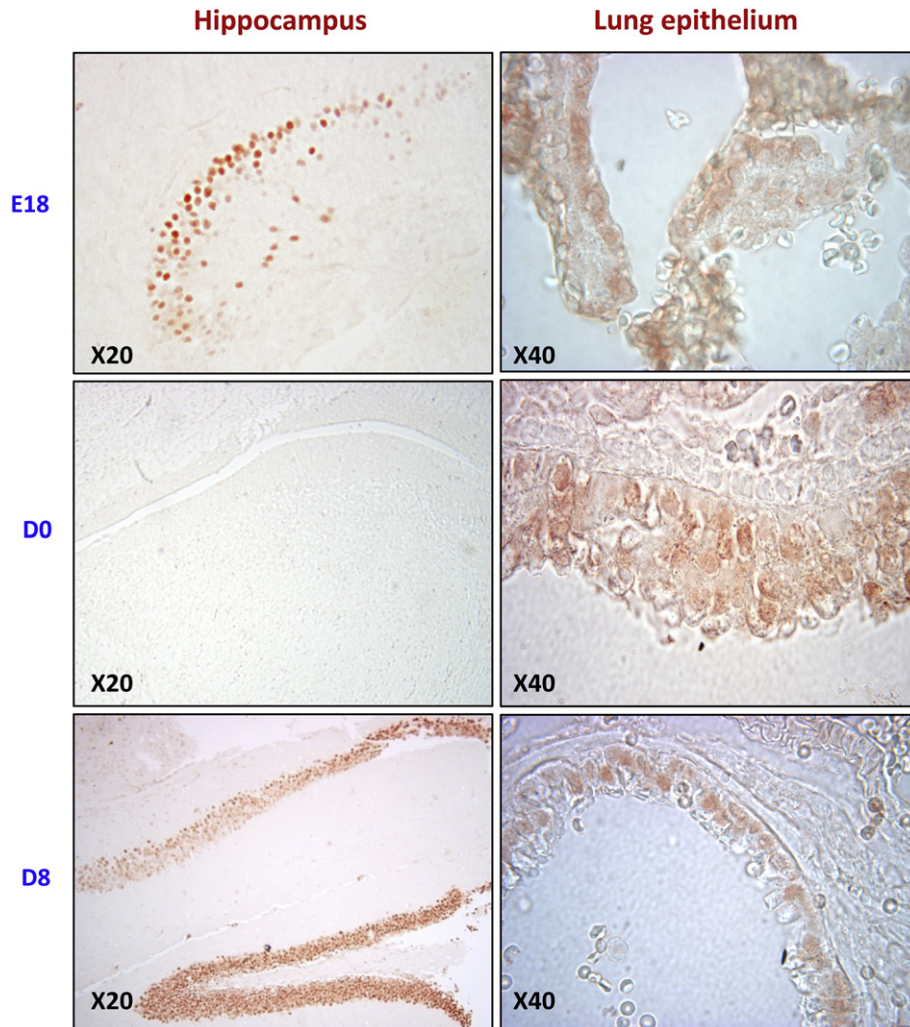


Fig. 3. Immunodetection of mineralocorticoid receptor (MR) protein in mice fetal and neonatal hippocampus (left panel) and pulmonary epithelia (right panel). MR is detected in the nucleus of hippocampus cells at 18 days of gestation (E18), and at 8 postnatal days (D8), but its expression is downregulated at birth (D0). In lungs, MR protein is present in the nuclei of epithelial cells at all developmental stages. **Methods:** wild-type mouse brain and lungs were collected from E18, D0 and D8 fetus and neonatal pups and fixed in buffered formol and embedded in paraffin for immunohistochemistry. Animal housing and experiments were conducted according to the French legislation. Five-micrometer-thick tissue sections were deparaffinized and rehydrated in successive baths of toluene and graded alcohols and subjected to 15 min microwave antigen retrieval in citrate buffer (pH 6.0). After cooling and 15 min preincubation with a blocking serum (Vector Laboratories, Burlingame, CA), slides were incubated with primary antibodies overnight at 4 °C in a humid chamber. Bound immunoglobulins were revealed with the ImmPRESS antimouse Ig kit (Vector) according to the manufacturer's instructions. MR monoclonal antibody [140] was used at dilution 1:120. Original magnification: $\times 20$ for hippocampus, $\times 40$ for pulmonary epithelium.

wild type control blastocysts, in order to study MR expression and function during early cardiac differentiation [127]. We showed that MR expression increases along cardiomyocyte differentiation in parallel with specific cardiac genes such as the early marker *Nkx2.5* and the late markers α MHC and troponin T. Interestingly, we were able to demonstrate a positive correlation between MR expression and the expression of the pacemaker channel *HCN1* which tightly controls the initiation of the depolarization process thereby modulating cardiomyocyte contractibility. Accordingly, it is likely that ES cell-derived cardiomyocytes represent a very early stage of cardiac differentiation and thus constitute a useful cell-based system to further evaluate MR function in early cardiac development. It could also be a useful model to elucidate the mechanisms generating cardiac diseases in adulthood [128,129]. Although MR knockout mice do not exhibit any gross cardiac abnormalities, it is well established that MR exerts a central role in the regulation of cardiomyocyte contractility and hypertrophy [127,130,131].

Thus, a better understanding of MR and GR regulation and expression during cardiomyocyte development could be of major interest in physiology and pathophysiology and could uncover novel therapeutic targets in cardiovascular diseases.

2.4. Skin

Even though very limited information is available concerning MR expression during skin development, it has recently arisen as another interesting MR target tissue and corresponds to the first report of an important developmental function for MR [56,57]. Mouse models of conditional MR-overexpression in keratinocytes have been generated in order to obtain a temporal control of transgene expression [56]. When MR was overexpressed throughout embryonic life, pups died shortly after birth presenting with skin abnormalities characterized by epidermal atrophy due to keratinocyte apoptosis starting at E18. This study demonstrates that overactivation of MR signaling has major consequences during

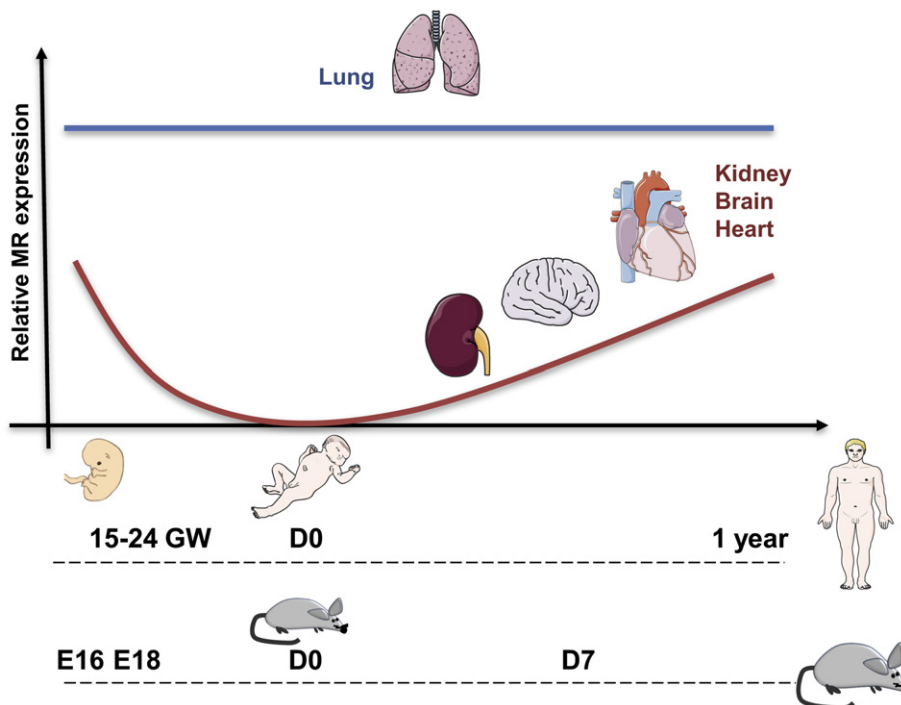


Fig. 4. Schematic representation of mineralocorticoid receptor (MR) ontogenesis in mice and humans. Relative MR expression levels are indicated in several target tissues during gestation, at birth and postnatally. Note that MR expression is downregulated during the prenatal period and especially at birth in the kidney, the brain and the heart while MR expression remains constant in the fetal and neonatal lung during the pre- and post-natal period.

skin development. MR knockout models die shortly after birth of dehydration due to severe salt wasting [33], which precludes obtaining information on the consequence of post-natal MR ablation in the skin. However, skin keratinization occurs around E17 during gestation [132]. Since MR knockout fetuses do not die during development, it appears that MR does not trigger skin keratinization but could exert its role rather in the modulation of keratinocyte proliferation. There again, the MR/GR balance could have a prominent role.

2.5. Lung

Lung is another important target tissue for mineralocorticoid action during development. Indeed, MR is expressed in adult rat [45,133] and human lungs [134], but MR mRNA has also been detected in the fetal and neonatal calf lung during the pre- and postnatal period [135,136] where MR modulates lung fluid composition [137]. MR protein is also detected in the epithelia of human fetal lungs during early gestation, along with 11β HSD2 [39]. Very interestingly, physiologic MR expression in murine lungs follows a very different pattern than in the kidney, brain and heart, with no significant downregulation at birth (Fig. 3, right panel). Given that reabsorption of pulmonary fluid, necessary for newborns to adapt to extra-uterine life, is mainly controlled by ENaC [138,139], MR expression in the pulmonary epithelium at this specific period of development could suggest a crucial role of the mineralocorticoid signaling pathway for the transition from aquatic to aerial life.

In sum, it appears that MR is involved in various physiological and pathophysiological processes, most notably renal regulation of sodium and fluid homeostasis. Nevertheless, the multiplicity of MR target tissues, the putative lethal consequences of a complete lack of human MR expression *in utero* (suggested by the fact that it has never been described in humans) as well as the severity of

phenotypes of newborns with aldosterone deficiency or MR defects, highly suggest that the mineralocorticoid signaling pathway plays a crucial role during development, most notably during the neonatal period.

3. Conclusion

These different studies have highlighted a new exciting role for the mineralocorticoid signaling pathway. Detection of high aldosterone concentrations beginning with the first trimester of gestation until the postnatal period, strongly suggest an early activation of the renin–angiotensin–aldosterone system, which sharply contrasts with the delayed, biphasic and tissue-specific expression of the MR. Fig. 4 summarizes the tissue-specific MR expression profile in the kidney, the CNS, the heart, and the lungs. Considering rapid aldosterone signaling through non-genomic effects [20], it is tempting to speculate that several aldosterone actions may take place throughout gestation and at birth. On the other hand, MR expression seems to be tightly regulated in a time- and tissue-specific manner, submitted to complex regulatory control including differential transcriptional activation of alternative promoters, as an adaptation of the fetus from intra-uterine to extra-uterine life. The aldosterone–MR signaling pathway could therefore be functional in the neonatal lung to facilitate alveolar fluid reabsorption, while being absent from the kidney to prevent excessive sodium and water reabsorption and thus contributing to remove extracellular volume excess. Moreover, as the glucocorticoid pathway is readily functional at birth, variability of MR expression could favor the glucocorticoid–GR signaling pathway in non-epithelial tissues, where MR and GR have opposite effects, facilitating developmental programming [89].

Altogether, these new insights into the expression and regulation of the mineralocorticoid signaling pathway throughout fetal development illustrate its crucial implication at specific

developmental stages, most notably around the neonatal period. Alterations in the timing or regulation of this pathway could therefore lead to deleterious outcomes in the short and long-term. As a result, a better understanding of the regulatory mechanisms controlling aldosterone secretion and MR expression during development ought to improve the management of newborn and preterm infants and perhaps could help in preventing the emergence of several diseases in adulthood.

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