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Aldosterone, the endogenous ligand of the mineralocorticoid receptor (MR) in humans, is a steroid hormone that regulates salt and water homeostasis. Recently, additional pathophysiological effects in the renocardiovascular system have been identified. Besides genomic effects mediated by activated MR, rapid aldosterone actions that are independent of translation and transcription have been documented. While these nongenomic actions influence electrolyte homeostasis, pH and cell volume in classical MR target organs, they also participate in pathophysiological effects in the renocardiovascular system causing endothelial dysfunction, inflammation and remodeling. The mechanisms conveying these rapid effects consist of a multitude of signaling molecules and include a cross-talk with genomic aldosterone effects as well as with angiotensin II and epidermal growth factor receptor signaling. Rapid corticosteroid signaling via the MR has also been demonstrated in the brain. Altogether, the function of nongenomic aldosterone effects seems to be to modulate other signaling cascades, depending on the surrounding milieu.

Aldosterone, the endogenous mineralocorticoid in humans, was first isolated in 1953 as the last of the steroid hormones (Simpson et al., 1954). In the early years, the main focus of research lay on the long-term effects of aldosterone on sodium-potassium homeostasis and regulation of blood pressure in so called mineralocorticoid target organs like kidney, colon and salivary glands. These effects were reported to be "genomic", i.e. dependent on transcription and translation. It was discovered that aldosterone binds to the mineralocorticoid receptor (MR) in the cytoplasm, causing dissociation of chaperones and formation of MR dimers. These dimers then translocate into the nucleus and act as transcription factors to influence the expression of certain genes. Several genes were identified as being directly or indirectly regulated by aldosterone, for example Na^+-K^+-ATPase, ENaC via SGK1 and ROMK, all of them concerned with electrolyte and volume regulation (Beesley et al., 1998; Chen et al., 1999; Kolla et al., 2000).

New interest in aldosterone arouse, when its effects in non-classical MC targets like VSMC, endothelial cells and cardiomyocytes were detected, showing an involvement in pathophysiological processes in the renocardiovascular system. Clinical studies demonstrated that patients with congestive heart failure or after myocardial infarction benefited from addition of MR antagonists to their treatment regiment (Pitt et al., 1999; Pitt et al., 2003a; Pitt et al., 2003b). Furthermore, the frequency of an elevated renin to aldosterone ratio in patients with hypertension was shown to be much higher than previously expected, leading to a reevaluation of the importance of aldosterone in patients with hypertension (Fardella et al., 2000; Rossi et al., 2006; Connell et al., 2003). In the search for the pathomechanism responsible for these positive effects of MR antagonists, the pathophysiological function of aldosterone in the renocardiovascular system came under fierce scrutiny. It was found that aldosterone participates in inflammatory and remodeling processes in these tissues, leading to fibrosis, endothelial dysfunction and hypertrophy (Blasi et al., 2003; Brilla et al., 1992; Qin et al., 2003; Rocha et al., 2002; Young et al., 2003). Because the mechanisms of action and additional conditions required were not clear, researchers began to look into the different signaling pathways of aldosterone in more depth. These include the rapid nongenomic effects, which do not require transcription or translation of genes. After long debates about their actual existence and biological relevance, discussions about their receptor and signaling pathways followed. Only recently, after the new interest in the pathophysiological effects of aldosterone and after acknowledging that there is a cross-talk between genomic and nongenomic effects have the possible consequences of rapid aldosterone signaling pathways come into focus.

First evidence for nongenomic aldosterone signaling

After long-time neglect of rapid aldosterone effects on sodium and potassium excretion in the kidney (Ganong et al., 1958), nongenomic effects of aldosterone were first postulated by Moura and Worcel in the mid 1980s (Moura et al., 1984). Early cellular studies demonstrated enhanced sodium exchange in canine erythrocytes, ruling out a genomic mechanism because of lack of a nucleus (SPACH et al., 1964). These studies were followed by investigations on aldosterone-mediated electrolyte transport in human mononuclear leukocytes (Wehling et al., 1989b; Christ et al., 1993; Wehling et al., 1990). A rapid increase in sodium-proton-exchange was detected which was declared nongenomic because of its rapid kinetics and insensitivity to inhibitors of translation or transcription (Wehling et al., 1989a). These early experiments explored aldosterone-dependent changes in second messenger systems without emphasis on the biological consequences. Nongenomic aldosterone-mediated signaling events described included an elevation of intracellular Ca^{++}, IP3, DAG and PKC, PLC and cAMP (Christ et al., 1993; Christ et al., 1995b; Christ et al., 1999; Wehling et al., 1995; Wehling et al., 1996). Importantly, these effects were shown to occur not only in renal cells but also in VSMC and endothelial cells, i.e. cells of non-classical mineralocorticoid target organs (Christ et al., 1995a; Wehling et al., 1994; Wehling et al., 1995; Wehling et al., 1996).

Much controversy arouse about the receptor conveying these effects. Early studies suggest that the nongenomic effects are mediated by a membrane receptor distinct from the classical cytoplasmic MR. Arguments supporting this concept include the rise in Ca^{++} and cAMP found in cultured skin cells from MR knockout mice (Haseroth et al., 1999). Furthermore, antagonists against the classical MR like canrenone and spironolactone were not always able to inhibit the rapid effects of aldosterone (Good et al., 2002). In several studies, glucocortoicoids, which bind to the classical MR with an affinity comparable to that of aldosterone, could not elicit the same rapid effects as aldosterone (Doolan et al., 1996a). And last, aldosterone coupled to large molecules like BSA and therefore unable to cross the membrane rapidly, elicited the same rapid effects, favoring a membrane receptor (Le Moellic et al., 2004). Similar receptors have been proposed for other steroid hormones (Orshal et al., 2004; Zhu et al., 2003). However, there are several arguments against a structurally different MR responsible for nongenomic effects as well. Firstly, more flexible and water-soluble MR antagonists like RU28318 are able to inhibit the same nongenomic aldosterone effects that cannot be inhibited by spironolactone (Michea et al., 2005; Mihailidou et al., 2005). Secondly, Alzamora and collegues show in an elegant study that the lack of response to cortisol is due to 11β-HSD. 11β-HSD is an enzyme co-localized with the MR which converts cortisol into the biologically inactive cortisone that is unable to bind to the MR. Inhibition of 11β-HSD by carbenoxolone caused cortisol to exert similar rapid effects like aldosterone (Alzamora et al., 2000). And thirdly, in a heterologous expression system of HEK cells lacking classical MR, nongenomic effects involving ERK1/2 and JNK activation could only be induced after transient transfection with the classical MR. Cells lacking this receptor showed no rapid MAP kinase activation after incubation with aldosterone (Grossmann et al., 2005). Nevertheless, the same heterologous cell system revealed a rise in intracellular Ca^{++} that is independent of the classical MR (Grossmann et al., 2005). Overall, the existence of nongenomically mediated aldosterone effects that seem to be mostly dependent on the classical MR and activate a variety of second messengers in both classical and nonclassical MR target organs was established in the early phase of rapid aldosterone research (Tab.1). These studies were followed by more in depth investigations on the signaling cascades involved and the pathophysiological effects conveyed.

Nongenomic aldosterone effects in classical mineralocorticoid target organs

In classical mineralocorticoid target organs like kidney and colon, a rapid effect of aldosterone on intracellular pH is detectable; a transient acidification is followed by a significant alkalization in both MDCK and M1 cells (Gekle et al., 1996; Markos et al., 2005; Wehling et al., 1996). There is much evidence that the rise in pH is caused by a rapid increase in intracellular Ca^{++} followed by activation of the sodium-proton exchanger (NHE) (Doolan et al., 1996b; Oberleithner et al., 1987; Gekle et al., 2001; Markos et al., 2005). In MDCK cells, prerequisite for this process is a net entry of Ca^{++} from outside the cell and a plasma membrane proton conductance to stimulate the NHE (Gekle et al., 1996). As source for the enhanced NHE activity, an increase in proton affinity was identified. When inhibiting NHE activity by a sodium free environment, proton conductance activation was unmasked and aldosterone induced a membrane potential-dependent acidification in MDCK and M1 cells. As shown by pharmacological studies, this process relies on PKC and heterotrimeric G proteins (Gekle et al., 1997). Furthermore, Harvey et al. demonstrated that aldosterone-induced activation of PKCα leads to a rapid increase in intracellular calcium through verapamil-sensitive ion channels, which then activates the NHE (Doolan et al., 1998; Harvey et al., 2002). The increase in pH then causes activation of a pH sensitive potassium channel K_{ATP} and inhibition of Ca^{++}-dependent K^+-channels which lead to an increase in K^+-recycling to maintain the electrical driving force for amiloride-sensitive Na^+-absorption while Cl^--secretion is inhibited (Maguire et al., 1999). Overall, the increase in intracellular sodium causes a change in cell volume that can be monitored by atomic force microscopy (Schneider et al., 1997b). These effects are insensitive to actinomycin D or cycloheximide. Further signaling

molecules involved are the MAP kinases ERK1/2. Inhibitors of ERK1/2 phosphorylation are able to inhibit both the increase in intracellular Ca^{++} and the activation of NHE. Preventing the increase in intracellular Ca^{++} eliminates NHE stimulation but not ERK phosphorylation (Gekle et al., 2001). These results suggest that activation of NHE is mediated by an ERK-dependent increase in intracellular Ca^{++} in MDCK cells. Upstream of ERK1/2, aldosterone induces phosphorylation of the epidermal growth factor receptor (EGFR). Alternatively, PKC-induced activation of ERK1/2 followed by stimulation of NHE has also been demonstrated in other cell types (Markos et al., 2005). Several investigators identified c-src as the link between aldosterone-bound MR and MAP kinases often with EGFR as intermediate (Callera et al., 2005b; Grossmann et al., 2005; McEneaney et al., 2007). Activation of ERK1/2 was dependent on the classical MR but analogous to the progesterone and the estrogen receptor, deletion constructs containing only the MR domains E/F were sufficient (Grossmann et al., 2008). Additionally, involvement of heat shock protein 90, the chaperone of the classical MR, has been reported (Braun et al., 2004; McEneaney et al., 2007). Besides regulating the ubiquitous basolateral NHE1, aldosterone also decreases apical NHE3 activity in the medullary thick ascending limb of the rat via an ERK1/2-dependent pathway, ultimately leading to a decrease in $HCO3^-$ reabsorption (Watts, III et al., 2006). The c-src –EGFR-signaling does not only lead to activation of MAP kinases but also seems to be a key element of another signaling pathway linking aldosterone to PKD1 activation in M1-CCD cells (McEneaney et al., 2007). To summarize, in classical mineralocorticoid target organs like kidney and colon, rapid effects of aldosterone are well characterized to lead to activation of PKC, intracellular calcium, EGFR and ERK1/2 and NHE with some evidence that the classical MR participates in at least some of these effects. Thereby, changes in pH and Na^+ are achieved, affecting cell volume and pH sensitive transporters like K_{ATP} and K_{ca} channels. Possible physiological consequences are regulation of cell volume independent of pH or membrane potential independent of sodium reabsorption.

Nongenomic aldosterone effects in non-classical target tissues

Aside from their effects on ion transport, aldosterone and the MR play an important role in cardiovascular diseases like indicated by three major clinical trials with MR antagonists and several smaller studies. The earliest trial, the RALES study, demonstrated an improvement in mortality and morbidity of patients with severe congestive heart failure after additional treatment with spironolactone (Pitt et al., 1999). A similar benefit could be detected after eplerenone in patients with left ventricular dysfunction after myocardial infarction (EPHESUS study) and in patients in the 4E study (Pitt et al., 2003a; Pitt et al., 2003b). Detailed investigations about the possible mechanisms leading to the advantageous effects of MR antagonists revealed an involvement of aldosterone in endothelial dysfunction, inflammation, remodeling processes and induction of hypertrophy and fibrosis (Leopold et al., 2007; Nagata et al., 2006; Guo et al., 2005; Rocha et al., 2002). At present, it is not clear which cell type(s), i.e. cardiac fibroblasts, cardiomyocytes or cells from vessels (VSMC, endothelium) is/are primarily responsible for these deleterious effects. Although it seems clear that genomic effects are involved in the pathophysiological effects of aldosterone outlined above, a contribution of rapid nongenomic effects to support them is also likely.

Especially a role in furthering endothelial dysfunction which then triggers a cascade of pathophysiological mechanisms leading to aldosterone´s pathophysiological effects has been discussed (Tab.2). Overall, with invasive and noninvasive techniques, aldosterone causes a vasoconstriction of resistance arteries with an increase in systemic peripheral resistance and a reduction in cardiac output (Klein et al., 1964; Schmidt et al., 1999; Wehling et al., 1998). Accordingly, the forearm blood flow was reduced after local intra-arterial application of aldosterone both in healthy volunteers and patients with stabile chronic heart failure (Gunaruwan et al., 2005; Romagni et al., 2003). However, opposite effects and no effects of aldosterone per se have also been reported (Klein et al., 1964; Nietlispach et al., 2007). Reconciling these adverse results, Schmidt et al.

showed that the effects of aldosterone depend on the adrenergic state of the individuals and their health status, which influences the equilibrium of endothelium versus vascular smooth muscle cell action (Schmidt et al., 2006; Schmidt et al., 2001; Schmidt et al., 2003). While aldosterone enhances the NO release of the endothelium, it also causes contraction of the smooth muscle cells underneath (Schmidt et al., 2006; Schmidt et al., 2003). Therefore, in a state of endothelial dysfunction, aldosterone is likely to cause further increase in resistance, thereby mediating arterial hypertension (Schmidt et al., 2006). Experimentally, this thesis was tested by applying aldosterone to intact and endothelium-denuded aortic rings. As a result, attenuation of phenylephrine-mediated vasoconstriction caused by aldosterone via enhanced NO synthase activity in intact aortic rings could be reversed into a vasoconstriction mediated by smooth muscle cells in endothelium-denuded aortic rings (Liu et al., 2003). In endothelial cells, one possible mechanism for the enhanced NO production is an PI3 kinase and PKB/Akt-mediated stimulation of eNOS possibly by synergizing calcium-dependent eNOS phosphorylation at Ser 1179 (Mutoh et al., 2008). Another study demonstrated that inhibition of NO synthase plus aldosterone causes an increase in GFR and a fall in RPF in healthy volunteers (Schmidt et al., 2006). Likewise the inhibitory action of aldosterone on depolarization-mediated vasoconstriction in renal afferent arterioles could be mimicked by the NO donor sodium nitroprussid (Uhrenholt et al., 2003). Aldosterone-induced contraction of smooth muscle cells was investigated further and found to be mediated by PLC, intracellular Ca^{++}, PKC and the IP3 pathway (Arima et al., 2003; Arima et al., 2004). Paralleled by a dose-dependent contraction of VSMC, a PI3 kinase-mediated enhanced phosphorylation of the myosin light chain was detected, which could lead to a vasoconstriction in preexisting endothelial dysfunction (Gros et al., 2007). Furthermore, in rabbit renal arterioles, the dose-dependent vasoconstriction caused by aldosterone in the afferent and efferent arteriole seems to depend on L-type VDCC in case of the afferent arteriole and on T-type VDCC in the case of the efferent arteriole (Arima et al., 2003). Because the ex vivo effects could be inhibited by spironolactone, an involvement of the classical MR in these rapid effects of the vasculature is suggested (Liu et al., 2003; Uhrenholt et al., 2003; Gros et al., 2007). Overall, these results indicate that aldosterone influences the vascular tone via the classical MR depending on the balance between endothelial NO release and vascular smooth muscle cell contraction and therefore depending on the health status and the surrounding milieu.

Recently, special emphasis has been laid on the balance between aldosterone´s ability to produce NO and to activate reactive oxygen species (ROS) in the endothelium (Skott et al., 2006). On the one hand, aldosterone is able to induce production of NO by eNOS in endothelium (Liu et al., 2003; Mutoh et al., 2008). Under conditions of low oxygen tension and NaCl diet this leads to a vasodilation. On the other hand, aldosterone also stimulates the NADPH oxidase via c-src leading to increased ROS generation (Iwashima et al., 2008). The generation of ROS is especially marked under conditions of high oxygen tension, high NaCl diet or inflammation, producing superoxide and together with NO peroxynitrite (Beckman et al., 1990; Beckman et al., 1996). Under such circumstances, NO concentrations are not only reduced by peroxynitrite formation but also by uncoupling of eNOS through oxidation of tetrahydrobiopterin (Kuzkaya et al., 2003). Aldosterone promotes the uncoupling of NOS further by activating PP2A and thereby causing dephosphorylation of Ser1177 (Nagata et al., 2006). Additionally, aldosterone decreases the expression of glucose-6-phosphate dehydrogenase and thereby limits the amount of NADPH available for scavenging oxygen radicals (Leopold et al., 2007). In conclusion, in an unstressed situation, aldosterone would lead to production of NO and vasodilation together with a low production of superoxide that would be mostly transformed to H_2O_2 and small amounts of peroxynitrite. With increased stress, activation of NADPH oxidase, inhibition of glucose-6-phosphate dehydrogenase and uncoupling of NOS will lead to an increase in superoxide, H_2O_2, and peroxynitrite that can cause oxidative damage and vasoconstriction as well as activation of platelets because of low NO concentrations (Skott et al., 2006).

A rapid aldosterone-induced activation of NADPH oxidase with consecutive enhanced production of ROS has been further reported in VSMC, rat neonatal myocytes and mesangial cells. In cardiomyocytes, as a consequence of enhanced ROS formation, apoptosis has been described as well as activation of MMP-2 and MMP-9 with the possible induction of remodeling processes (Hayashi et al., 2008; Rude et al., 2005). Rapid activation of the NADPH oxidase depended on ERK1/2 phosphorylation and PKC and most likely involves enhanced membrane translocation of p47phox and p67phox as suggested by Miyata et al. (Miyata et al., 2005). The constant RNA levels found for NOX2 in cardiomyocytes support this hypothesis (Hayashi et al., 2008). Furthermore, c-src has also been accused of activating NADPH oxidase in VSMC. For c-src a rapid aldosterone-mediated activation of p38 which causes enhanced proline incorporation has been reported, indicating enhanced collagen synthesis and profibrotic actions. An upregulation of this pathway with increased c-src phosphorylation, NADPH oxidase activity and collagen synthesis could be found in vascular myocytes from spontaneously hypertensive rats and was sensitive to inhibition by eplerenone (Callera et al., 2005a). Because some genomic pathophysiological aldosterone effects are dependent on the coexistence of additional stressors like ROS, nongenomic ROS production could have a profound influence on the overall pathophysiological processes induced by aldosterone (Gekle et al., 2007).
An additional pathophysiological mechanism described for aldosterone is the induction of inflammation which then leads to remodeling processes including fibrosis and hypertrophy. Recent studies indicate that mineralocorticoid-induced NHE activation in vascular smooth muscle cells is involved in such inflammatory processes, measured as an increase in ED-1 and osteopontin. In the same study, enhanced collagen deposition was also detected, which could be inhibited by canrenoate or the NHE blocker cariporide (Young et al., 2003). Additionally, inhibition of NHE1 was able to prevent DOCA / salt-induced MR-mediated cardiac hypertrophy (Fujisawa et al., 2003). The mechanisms for this augmented NHE activity include an increase in Na^+ sensitivity but also an increase in NHE expression via enhanced translocation to the membrane and stimulation of de-novo synthesis (Ebata et al., 1999). Like demonstrated for classical MR target organs, the Na^+/H^+ exchange can therefore be nongenomically activated but it also possesses a prolonged component which is attributed to genomic actions (Miyata Y et al., 2005; Ebata et al., 1999). Taken together, the pathophysiological effects leading to inflammation and remodeling seem to involve classical MR and are mediated by a combination of nongenomic and genomic effects which interact with one another.

A further aldosterone-dependent pathway leading to cardiac remodeling with emphasis on hypertrophy is inhibition of the Na^+-K^+ ATPase (Kometiani et al., 1998). In a series of patch clamp studies involving rabbit cardiomyocytes, aldosterone caused a decrease in Na^+-K^+ pump activity and an increase in intracellular sodium activity after 7 days of treatment. This effect on the pump could be inhibited by losartan or MR inhibitors (Mihailidou et al., 2000; Mihailidou et al., 2002). Additionally, a rapid increase in Na^+-K^+-$2Cl^-$ cotransporter activity was found, leading to an increase in intracellular sodium with increase in Na^+-K^+ ATPase activity. When inhibiting the Na^+-K^+-$2Cl^-$ cotransporter, a decreased Na^+-K^+ ATPase activity could be unmasked that was mediated by PKCε. Interestingly, the long-lived and prolonged inhibitory effect of aldosterone on the pump could be inhibited acutely by inhibition of PKCε, again suggesting that genomic and nongenomic effects are intertwined (Mihailidou et al., 1998; Mihailidou, 2006). Besides having direct effects on cardiomyocytes, aldosterone can also indirectly enhance cardiac remodeling by reducing compensatory mechanisms in response to stressors. For example, the intracoronary administration of aldosterone rapidly decreased coronary blood flow along with fractional shortening and lactate extraction rate. This decrease in coronary blood flow was reproduced by the infusion of bovine serum albumin-conjugated aldosterone and blunted by co-administration of a PKC inhibitor, indicating that aldosterone nongenomically induces vasoconstriction via a PKC-dependent pathway. In case of ischemia, this mechanism leads to a worsening of the cardiac contractile and metabolic

functions and therefore to an increase in cardiac damage (Fujita et al., 2005). Thus, aldosterone can augment cardiac remodeling through direct effects on the cardiomyocytes transporters but also through indirect effects that decrease the hearts ability to cope with injuries. Paradoxically, conditional overexpression of MR in cardiomyocytes causes arrhythmias but does not induce structural alterations in the heart while partial knock-down of the MR results in severe cardiac failure and fibrosis (Beggah et al., 2002; Ouvrard-Pascaud et al., 2005).

Interaction between nongenomic and genomic

As demonstrated above, there are indications for an interaction between nongenomic and genomic aldosterone signaling throughout literature with a tendency that nongenomic effects enhance or support genomic effects. For instance, by measuring the genomic response to aldosterone with and without inhibiting rapid ERK1/2 activation, one finds that nongenomic ERK activation enhances genomic aldosterone responses at a glucocorticoid response element. As a possible mechanism for this interaction an induction of the nuclear-cytoplasmic shuttling was identified (Grossmann et al., 2005). Interestingly, this interaction between nongenomic and genomic aldosterone effects is dependent on the n-terminal domain of the MR; deletion constructs without A/B domain do not respond to inhibition of the rapid ERK1/2 signaling. Furthermore, inhibiting the early aldosterone-induced PKCα pathway results in reduced MR transactivation activity and short-circuit current (Le Moellic et al., 2004). A rapid aldosterone- and PKCα-mediated phosphorylation and thereby activation of the MR was demonstrated as the underlying mechanism. Several reports indicate synergistic effects of nongenomic and genomic aldosterone actions. For example, activation of NHE that induces pathophysiological effects like vasoconstriction, cardiac fibrosis and hypertrophy (Fujisawa et al., 2003; Michea et al., 2005; Young et al., 2003) is regulated nongenomically and genomically by aldosterone and PKC in VSMC (Ebata et al., 1999; Miyata Y et al., 2005). Moreover, some of the nongenomic effects begin rapidly but continue as prolonged effects like indicated by the long-lived effect of aldosterone on the Na^+-K^+-ATPase that could be rapidly reversed by PKCε inhibition within 15 min (Mihailidou et al., 2004a). Other indications for the importance of nongenomic effects for long lasting effects come from in vivo studies that suggest that nongenomic aldosterone effects lead to an increase in renal vascular resistance with a decrease in GFR and an increase in RPF in humans with impaired endothelial function (Schmidt et al., 2006). Consequently, nongenomic aldosterone signaling can lead to long-lasting effects either by being prolonged itself or by interacting with genomic effects. Another intriguing finding is that aldosterone exerts nongenomic effects on the same ion transporters that it also regulates genomically. A possible mechanism for the nongenomic aldosterone effects is an interaction with the cytoskeleton and thereby with the trafficking of ion transporters. For example, aldosterone rapidly activates the NHE in VSMC, a process that can be blocked by inhibitors of microtubule polymerization and filamentous actin formation (Ebata et al., 1999). A similar effect of aldosterone on the cytoskeleton has been documented for the epithelial sodium channel ENaC. McEneamy et al. showed that aldosterone nongenomically influenced the trafficking of this channel (McEneaney et al., 2008). Additionally, ENaC activation could be inhibited by colchicine and cytochalasin D (Golestaneh et al., 2001). A similar inhibitory effect of colchicine on the nongenomic aldosterone actions has also been described for the sodium-potassium-pump although in this case the nongenomic actions antagonized the genomic effect (Alzamora et al., 2003). Taken together, there are various interactions between nongenomic and genomic aldosterone effects including direct facilitation of genomic signal transduction through nongenomic signaling and synergistic effects of both mechanisms of action on certain target molecules. The demonstration of prolonged nongenomic aldosterone effects highlights their relevance for overall aldosterone actions but also renders the distinction between genomic and nongenomic signaling more difficult.

Interaction with other signaling molecules: angiotensin II, EGFR

However, there is not only a cross-talk between nongenomic and genomic pathways of aldosterone but also with other pathophysiologically relevant signaling cascades of the renocardiovascular system. One of these signaling partners is angiotensin II (angII) which classically binds to the G-protein-coupled AT1 receptor, thereby leading to aldosterone secretion from the adrenals. Joint actions between MR and angII have been described in cells that are incapable of secreting aldosterone. For instance, in aortic rings from Sprague-Dawley rats and in human coronary arteries, aldosterone enhanced angII-stimulated contractions (Chai et al., 2005; Ullian et al., 1996). Early observations demonstrate further that angII-induced incorporation of ^{3}[H]-thymidine in rat aortic smooth muscle cells can be inhibited by spironolactone and that aldosterone is required for full proliferative response to angII. In neither case, aldosterone had an effect per se. One suggested interaction was a modulation of AT1 receptor expression (Xiao et al., 2000; Xiao et al., 2004). Experiments in human coronary smooth muscle cells, however, reveal that angII via the AT1R enhances translocation of the MR into the nucleus with resulting alterations in gene expression, suggesting a transactivation of the MR via the AT1R (Jaffe et al., 2005). While these effects are not necessarily nongenomic, Mazak et al. showed that aldosterone and angII lead to a potentiation of rapid ERK1/2 and JNK phosphorylation in VSMC of double transgenic human renin and angiotensin rats that could not be inhibited by actinomycin D or cycloheximide. MR blockade reduced ERK1/2 phosphorylation and attenuated angII-induced end-organ damage in vivo (Mazak et al., 2004). This observation was carried further by Min et al., who found that aldosterone and angII synergistically induce an ERK-dependent mitogenic response in VSMC measured as enhanced DNA synthesis (Mazak et al., 2004; Min et al., 2005). This effect was attributed to aldosterone actions mediated by nongenomic and genomic mechanisms.

Phosphorylation of ERK1/2 is one of the best documented signaling steps of nongenomic aldosterone actions (Gekle et al., 2001; Mazak et al., 2004; McEneaney et al., 2007; Rossol-Haseroth et al., 2004). Besides being part of a cascade that includes upstream activation of PKC, ERK1/2 is also part of epidermal growth factor receptor (EGFR) signaling (Grossmann et al., 2005; Markos et al., 2005). The EGFR is a growth factor receptor known for its induction of cell proliferation and remodeling processes in the renocardiovascular system (Francois et al., 2004; Kagiyama et al., 2003; Terzi et al., 2000) and it is also responsible for at least part of the pathophysiological effects induced by angII and endothelin-1 in these tissues (Bokemeyer et al., 2000; Flamant et al., 2003; Kagiyama et al., 2002; Zhai et al., 2006). Several reports exist about an interaction between aldosterone and the EGFR. They include a spironolactone-induced decrease in EGFR mRNA and protein that coincides with a decrease in cerebral vascular remodeling and infarct size or a decrease in perivascular fibrosis in coronary arteries (Dorrance et al., 2001; Dorrance et al., 2006; Nakano et al., 2005). Additionally, an increase in EGFR mRNA and an upregulation of arterial contraction to epidermal growth factor was elicited by aldosterone (Florian et al., 2001). While aldosterone-bound MR enhances EGFR promoter activity and consequently EGFR expression, transactivation of the EGFR has also been found (Grossmann et al., 2007; Grossmann et al., 2005). As a bridge molecule between aldosterone-activated MR and transactivation of the EGFR c-src was identified (Grossmann et al., 2005; McEneaney et al., 2007). Interestingly, overexpression of MR without steroids also leads to ligand-independent ERK1/2 activation (Grossmann et al., 2008). One consequence of rapid aldosterone actions is a sensitization of cells for EGF with a leftward shift of the dose-response curve in the presence of the EGFR (Krug et al., 2002). Furthermore, through the EGFR pathway, Na^{+}-H^{+}-exchanger activity is stimulated and PKD activated (Gekle et al., 2002; McEneaney et al., 2008), which eventually results in ENaC subunit intracellular trafficking affecting sodium content of cells and proliferation of cardiomyocytes. Nongenomic aldosterone effects also contribute to proliferation of vascular smooth muscle cells via phosphorylation of the EGFR (Min et al., 2005). Together with angII, aldosterone leads to a rapid and

sustained ERK phosphorylation mediated by EGFR transactivation which then leads to changes in gene expression of ki-ras2A and downregulation of the phosphatase MkP-1 which is responsible for proliferation of VSMC. Additionally, stimulation of cardiac fibroblast proliferation has also been associated with ki-rasA induction (Stockand et al., 2003). To decipher between prolonged nongenomic and genomic effects, deletion constructs of the MR without DNA-binding domain were tested for their effect on collagen III secretion. Even without DNA-binding domain, aldosterone-induced MR furthered H_2O_2-stimulated increase in collagen III secretion. This seems to be a prolonged nongenomic effect involving EGFR, highlighting that nongenomic aldosterone effects can have long-lasting results with pathophysiological relevance (Grossmann et al., 2008; Mihailidou et al., 2004b). Overall, the EGFR is an important signaling component that appears in genomic and nongenomic signaling of aldosterone and takes part in the cross-talk between both ways of communication.

Nongenomic corticosteroid effects in the brain

Another non-classical target tissue involved in rapid mineralocorticoid receptor effects is the brain. While some parts of the CNS that regulate salt appetite have access to aldosterone and possess 11β-HSD to ensure MR specificity, most parts of the brain possess MRs without specificity. Importantly, in these areas glucocorticoids possess two signaling pathways either via the high affinity MR or the lower affinity GR. Rapid corticosteroid effects have been described on feedback operation in the HPA axis (Dallman, 2005), in appraisal of novel situations and retrieval processes (Di et al., 2003; Oitzl et al., 1994). For example, a rapid and therefore nongenomic negative feedback control of corticosterone on the production and release of CRH was demonstrated. As a possible mechanism, corticosterone was shown to decrease the release probability of glutamate-containing vesicles in the paraventricular nucleus (Di et al., 2003). This presynaptic process was dependent on a postsynaptic G-protein linked pathway (Malcher-Lopes et al., 2006). Furthermore, endocannaboinoid synthesis and signaling via a presynaptic CB1 receptor were suggested to be involved (Di et al., 2003). Neither classical MR nor GR seem to mediate this effect.

Especially high levels of MR expression are located in limbic areas, mainly the hippocampus. Rapid effects of aldosterone on the hippocampus include facilitation of the release of glutamate containing vesicles thus enhancing miniature excitatory postsynaptic currents in CA1 neurons via a presynaptic ERK1/2 pathway (Olijslagers et al., 2008). This effect can be blocked by spironolactone and is not present in forebrain-specific MR knockout mice receiving corticosterone (Karst et al., 2005). Additionally, a decrease in K^+-conductance IA via postsynaptic G-protein-coupled MRs was found, possibly increasing the likelihood of a postsynaptic action potential further (Olijslagers et al., 2008). One consequence could be the facilitation of long-term potentiation which has been described as a long-term effect of activated MR (Avital et al., 2006; Berger et al., 2006). Furthermore, a rapid effect of corticosterone in facilitating long-term potentiation of beta-adrenergic agonists has been described as well as a rapid MAP kinase- and MR-dependent maintenance of long-term potentiation by swim stress in the dentate gyrus (Ahmed et al., 2006; Korz et al., 2003; Pu et al., 2007). Overall, speculations have been made by de Kloet et al that the permissive effects of corticosterone, partly mediated by MR, may further rapid encoding of stress-related information and selecting of appropriate behavioral responses for coping (de Kloet et al., 2008). On the other hand, there are also reports about a nongenomic blockade of memory retrieval by activated MR (Khaksari et al., 2007). Overall, the function of the MR in the CNS is complex and needs to be elucidated further.

In summary, nongenomic aldosterone signaling consists of an intricate network of signaling cascades that often involve the classical MR (Fig. 1). In classical MC target organs like kidney and colon, rapid effects of aldosterone have been demonstrated to influence electrolyte homeostasis, pH and cell volume. Additionally, pathophysiological effects including inflammation, remodeling processes and endothelial dysfunction have been described that have been especially well documented in cells of

the renocardiovascular system. Nongenomic aldosterone actions support these effects by modulating vascular tone, ROS production, remodeling processes and proliferation of cells, making tissues more vulnerable to additional stress. As mechanisms of action, a combination of nongenomic and genomic effects that interact with one another have been identified. However, aldosterone not only possesses a cross-talk between its own signaling pathways but it also modulates the signaling pathways of other pathophysiologically relevant molecules like angII and the epidermal growth factor receptor (EGFR). Corticosteroids in the brain also exert nongenomic effects and are involved in neuronal function and stress response via the MR.

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Fig. 1
Overview over the different pathways and effects of aldosterone signaling.

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Tab. 1 Molecules involved in nongenomic aldosterone signaling

cAMP	porcine VSMC Inner medullary collecting duct	(Christ et al., 1999) (Sheader et al., 2002)
IP3	HML VSMC A6 cells skeletal muscle cells	(Christ et al., 1993) (Christ et al., 1995) (Blazer-Yost et al., 1999) (Estrada et al., 2000)
Ca^{2+}	VSMC, EC VSMC (rat and rabbit aortic) T84 colonic epithelial cells porcine EC skin fibroblasts from MR−/− mice skeletal muscle cells M-1 cells small resistance mesenteric vessels HEK (MR) cells	(Wehling et al., 1994) (Wehling et al., 1995) (Doolan et al., 1996) (Schneider et al., 1997) (Haseroth et al., 1999) (Estrada et al., 2000) (Harvey et al., 2000) (Michea et al., 2005) (Grossmann et al., 2005)
PKC	rat VSMC MDCK rat VSMC M1 cells rabbit cardiomyocytes rabbit preglomerular afferent arteriole OMCD coronary arteries (LAD) dogs adult rat ventricular cardiomyocytes human trabeculae (heart) in vitro crypt cells from human sigmoid colon	(Estrada et al., 2000) (Gekle et al., 1997) (Ebata et al., 1999) (Harvey et al., 2000) (Mihailidou et al., 2004a) (Arima et al., 2004) (Winter et al., 2004) (Fujita et al., 2005) (Rude et al., 2005) (Chai et al., 2005) (Alzamora et al., 2007) (Bowley et al., 2007)
ERK1/2	MDCK C11 M1 cells HEK, CHO cells: heterolog system aortic VSMC (rat) aortic VSMC (rat) adult rat ventricular cardiomyocytes rat aortic smooth muscle cells human coronary arteries M1 cells rat MTAL bovine aortic endothelial cells, rat VSMC ERK phosporylation M1 cells rat mesangial cells HK-2 cells	(Gekle et al., 2002) (Rossol-Haseroth et al., 2004) (Grossmann et al., 2005) (Mazak et al., 2004) (Min et al., 2005) (Rude et al., 2005) (Ishizawa et al., 2005) (Chai et al., 2005) (McEneaney et al., 2007) (Watts, III et al., 2006) (Liu et al., 2003) (Kobayashi et al., 2006) (Markos et al., 2005) (Terada et al., 2005) (Zhang et al., 2007)
PI3K	small mesenteric resistance vessels rat human VSMC rabbit renal afferent arteriole bovine aortic endothelial cells rat left ventricle Heart bovine aortic EC	(Michea et al., 2005) (Gros et al., 2007) (Uhrenholt et al., 2003) (Liu et al., 2003) (Kobayashi et al., 2006) (Mutoh et al., 2008)
c-src	M1 cells rat VSMC rat VSMC (SHR) rat VSMC HEK (MR) cells	(McEneaney et al., 2007) (Callera et al., 2005b) (Callera et al., 2005a) (Montezano et al., 2008) (Grossmann et al., 2005)
interaction angII	rat aortic VSMC rat aortic VSMC human coronary arteries rat VSMC human heart trabecula human coronary artery smooth muscle cells dTGR rats, left ventricle dTGR rats,heart kidney rat mesenteric small arteries, aortic segments MR overexpression in cardiomyocytes (mice)	(Mazak et al., 2004) (Min et al., 2005) (Chai et al., 2005) (Montezano et al., 2008) (Chai et al., 2005) (Jaffe et al., 2005) (Fiebeler et al., 2001) (Fiebeler et al., 2005) (Virdis et al., 2002) (Di Zhang et al., 2008)
interaction EGFR	MDCKs-C11 cells rat VSMC M11 cells HEK-MR, CHO-MR cells rat aortic VSMC	(Gekle et al., 2002) (Mazak et al., 2004) (McEneaney et al., 2007) (Grossmann et al., 2005) (Min et al., 2005)
NADPH oxidase / ROS	RAEC rat mesangial cells neonatal rat cardiomyocytes rat VSMC adult rat ventricular myocytes rat aorta rat VSMC HK-2 cells	(Iwashima et al., 2008) (Miyata et al., 2005) (Hayashi et al., 2008) (Montezano et al., 2008) (Rude et al., 2005) (Virdis et al., 2002) (Mazak et al., 2004) (Zhang et al., 2007)
NOS	HUVEC bovine aortic EC rabbit renal afferent arteriole bovine aortic EC rabbit afferent arteriole kidney rat myocardial slices	(Nagata et al., 2006) (Mutoh et al., 2008) (Uhrenholt et al., 2003) (Liu et al., 2003) (Arima et al., 2004) (Kobayashi et al., 2006)
NHE	giant cells of distal nephron of frog MDCK small resistance mesenteric vessels rat VSMC VSMC MTAL M1 cells Chorionic / radial uterine arteries HML	(Oberleithner et al., 1987) (Gekle et al., 1996) (Chai et al., 2005) (Ebata et al., 1999) (Miyata Y et al., 2005) (Good et al., 2006) (Markos et al., 2005) (Alzamora et al., 2000) (Wehling et al., 1989)

Tab. 2 Nongenomic aldosterone effects in cardiomyocytes and vessels

cardiomyocytes		(Mihailidou et al., 1998) (Mihailidou et al., 2000) (Mihailidou et al., 2004b) (Barbato et al., 2002) (Chai et al., 2005) (Hayashi et al., 2008)
vasculature **human volunteers / patients**		(Nietlispach et al., 2007) (Klein et al., 1964) (Schmidt et al., 2001) (Romagni et al., 2003) (Schmidt et al., 2003) (Schmidt et al., 2006) (Gunaruwan et al., 2005) (Schmidt et al., 1999) (Wehling et al., 1998)
vasculature **experimental data**	**vasodilation**	(Uhrenholt et al., 2003) (Liu et al., 2003)
	vasoconstriction	(Arima et al., 2003) (Chai et al., 2005)

Fig. 1