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Nongenomic effects of mineralocorticoid receptor activation in the cardiovascular system

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Abstract

Fifteen years ago Wehling and colleagues showed unequivocal rapid effects of aldosterone, neither mimicked by cortisol nor blocked by spironolactone, and postulated that these nongenomic effects are mediated via a membrane receptor distinct from the classical mineralocorticoid receptor (MR). Several recent studies have challenged this view. Alzamora et al. showed 11β-hydroxysteroid denydrogenase 1 and 2 (11βHSD1, 11βHSD2) expression in human vascular smooth muscle cells, and that aldosterone rapidly raises intracellular pH via sodium–hydrogen exchange; cortisol is without effect and spironolactone does not block the aldosterone response. When, however, 11βHSD activity is blocked by carbenoxolone, cortisol shows agonist effects indistinguishable from aldosterone; in addition, the effect of both aldosterone and cortisol is blocked by the open E-ring, water soluble MR antagonist RU28318. In rabbit cardiomyocytes, aldosterone increases intracellular [Na^+] by activating $Na^+/K^+/2Cl^-$ cotransport, with secondary effects on Na^+/K^+ pump activity. Pump current rises ∼10-fold within 15′, is unaffected by actinomycin D or the MR antagonist canrenone, and not elevated by cortisol. Pump current is, however, completely blocked by the open E-ring, water soluble MR antagonist K^+ canrenoate and stoichometrically by cortisol. PKCε agonist peptides (but not PKCα, PKCδ or scrambled PKCε peptides) mimic the effect of aldosterone, and PKCε antagonist peptides block the effect. Very recently, cortisol has been shown to mimic the effect of aldosterone when cardiomyocyte redox state is altered by the installation of oxidized glutathione (GSSG) via the pipet, paralleling the effect of carbenoxolone on vascular smooth cells and suggesting possible pathophysiologic roles for an always glucocorticoid occupied MR.

Keywords: Steroid; Aldosterone; Mineralocorticoid receptors; Cortisol; Redox state; 11βHSD2

1. Background

In studies over the past 15 years, Wehling and colleagues showed that aldosterone at nanomolar concentrations produced rapid, nongenomic effects in a variety of tissues (reviewed in [1]). In one such study [2], the effect of a range of aldosterone concentrations on intracellular [Ca^{2+}] in human vascular smooth muscle cells can be seen to reach plateau levels in 2–3 min (Fig. 1, top panel). Secondly, in the same study, the relative potencies of a range of steroids was established (lower panel). Aldosterone, 9αfluorocortisol and deoxycorticosterone were shown to be agonists, with EC_{50} values ranging from 0.1 to 3 nM; importantly, cortisol showed no agonist activity at doses 1000-fold higher than those of aldosterone, and in addition spironolactone at high concentrations did not block the rapid aldosterone effect (not shown).

On the basis of these and similar activity findings Wehling proposed that such rapid nongenomic effects of mineralocorticoids were mediated by activation of a membrane receptor distinct from the classical nuclear transcription factor first cloned in Ron Evans' laboratory [3]. Subsequent attempts to isolate and characterize a membrane receptor for aldosterone have proved to be unrewarding, although a seven transmembrane domain membrane receptor for progesterone, clearly distinct from the classical intracellular progesterone receptor, has recently been cloned and characterized in a variety of species [4].

Over the same period, however, evidence has been forthcoming that rapid nongenomic effects of aldosterone on

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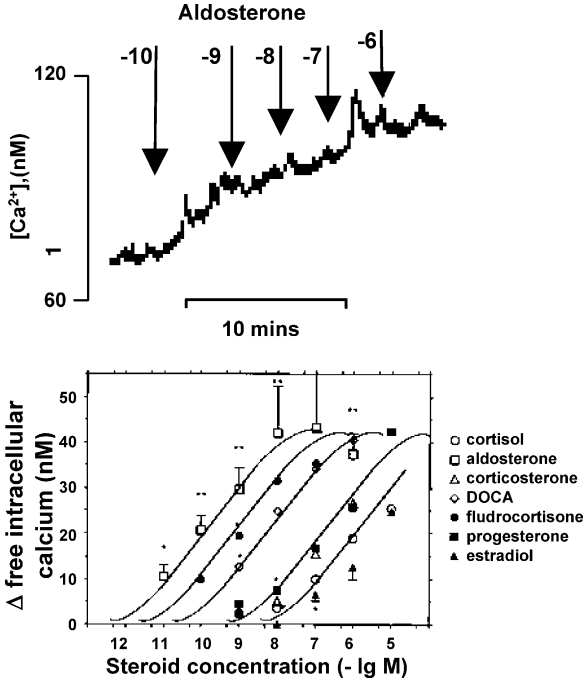

Fig. 1. Rapid nongenomic effect of aldosterone on Ca^{2+} flux in vascular smooth muscle cells. Upper panel: dose-dependence of aldosterone effect. Lower panel: relative potency of naturally occurring and synthetic steroids. Adapted from [2].

vascular smooth muscle cells [5] and cardiomyocytes [6,7] are mediated via classical mineralocorticoid receptors (MR). This does not exclude rapid effects of aldosterone being mediated by a quite distinct membrane receptor as exemplified by the distinct progesterone receptors. Indeed, aldosterone effects on intracellular Ca^{2+} flux have been reported in cultured skin cells from MR null (−/−) mice [8]; the physiologic importance of this demonstration is uncertain, in that skin the response in cells from knockout mice is almost an order of magnitude higher than in wild-type.

1.1. Specificity of aldosterone action via classical MR

In contrast with keratinocytes, vascular smooth muscle cells are a physiologic target for aldosterone, in that they express both MR and the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD) [9], and show effects that can be both mimicked by similar concentrations of cortisol under defined conditions and blocked by open E-ring MR antagonists [5]. How these studies, and those on cardiomyocytes [6,7], can be construed as supporting rapid actions of aldosterone via MR, however, requires a brief review of the mechanisms that allow aldosterone to selectively activate MR in target tissues.

Studies on cytosol preparations of tissues from adrenalectomized rats [10] and subsequent studies on the human MR [3] raised two particular questions. The first was the finding of MR in heart and hippocampus, for example, neither of which is a classic aldosterone target tissue. Secondly, both rat and human MR showed the same high affinity for aldosterone, corticosterone and cortisol. Circulating total levels of physiologic glucocorticoid are ∼1000-fold higher than those of aldosterone, and plasma free levels ∼100-fold higher, posing the question of how aldosterone can access MR in mineralocorticoid target tissues.

Possible roles for MR in tissues such as cardiomyocytes and neurons will be proposed later in this paper. In terms of specifying aldosterone occupancy and activation of epithelial MR, the answer appears to be the coexpression of the enzyme 11βHSD2 in aldosterone target tissues. Initially it was proposed [11,12] that 11βHSD2 converted intracellular cortisol to cortisone, which has very low affinity for MR; aldosterone is not similarly metabolized, as its C11 hydroxyl cyclizes with the signature C18 aldehyde group, forming an 11,18 hemiacetal and thus protecting it against enzymatic attack.

While these studies were interpreted as epithelial MR being protected against occupancy by endogenous glucocorticoids, in fact the enzyme protects against activation by glucocorticoids. This is a crucial difference and reflects the demonstration [13] that the enzyme 'debulks' intracellular glucocorticoid levels, reducing them from ∼100-fold those of aldosterone to ∼10-fold (Fig. 2). This means that when the enzyme is operating most epithelial MR are occupied but not activated by glucocorticoids. When the enzyme is deficient, as in the syndrome of apparent mineralocorticoid excess, or blocked by licorice abuse or by carbenoxolone, glucocorticoid–MR complexes are activated, leading to uncontrolled sodium retention and severe hypertension. This reflects not a further increase in MR occupancy by cortisol, but precipitous lowering of NADH levels in the vicinity of the MR; in other systems [14,15] NADH has been shown to activate corepressors, and changes in intracellular redox state have been invoked in a variety of circumstances to activate MR–glucocorticoid complexes [16–18].

1.2. Acute effects of aldosterone on vascular smooth muscle cell

In vitro studies from Marusic's laboratory [5] showed human vascular smooth muscle cells to express 11βHSD1 and 11βHSD2, and in addition that intracellular pH in such cells could be acutely (<5 min) raised by nanomolar concentrations of aldosterone. This effect is mediated by acute activation of the Na^+/H^+ exchanger and can be blocked by the amiloride derivative EIPA. In keeping with the studies from the Wehling laboratory, the pH effect of aldosterone is not affected by added spironolactone; in contrast, however, it is completely blocked by the water soluble, open E-ring MR antagonist RU28316 (Fig. 3, top).

Again, as was the case for previous studies on the rapid effects of aldosterone, cortisol alone is without discernible effects. When, however, the cortisol-metabolizing, NADH-generating enzyme 11βHSD2 is blocked by carbenoxolone, cortisol mimics the aldosterone action on intracellular pH, as shown in the lower panel of Fig. 3. This response would appear to underline two important features of MR

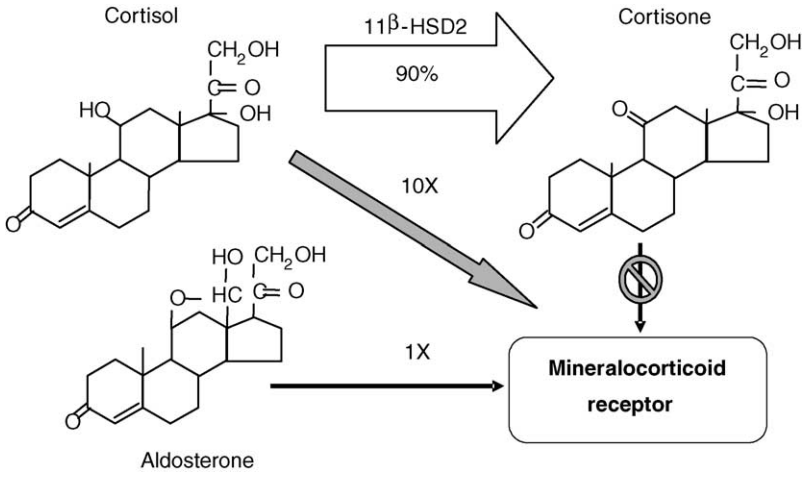

Fig. 2. Cartoon of a mineralocorticoid target tissue cell and functioning of 11βHSD2 to protect the MR, by debulking intracellular cortisol concentrations and by generating NADH to inactivate glucocorticoid occupied MR.

activation in these cells. First, the initial concentration of cortisol (1 nM) is in no way 'excess' levels and its very rapid effect suggests a preexisting inactive glucocorticoid–MR conformation, activation of which reflects the acutely changed redox status of the cell. Secondly, whereas the Na^+/H^+ exchanger is plasma membrane located, 11βHSD is largely tethered in the endoplasmic reticulum. The effects of 11βHSD blockade on NADH levels may extend from ER to cell membrane, or may be more compartmentalized. To the extent that the latter is the case, it argues for a cytoplasmic location of MR being able to produce acute nongenomic effects, rather than a necessarily membrane location.

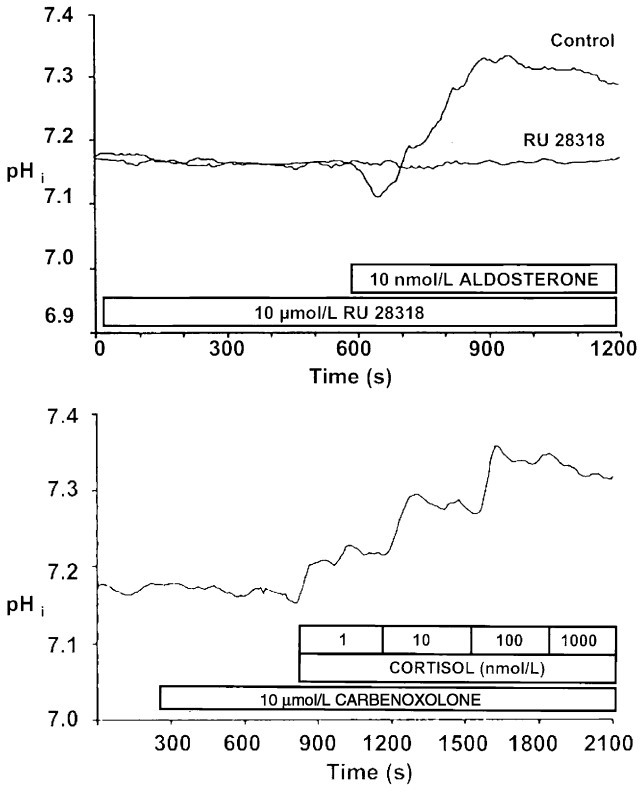

Fig. 3. Rapid nongenomic effect of aldosterone on intracellular pH in vascular smooth muscle cells. Upper panel: abrogation of aldosterone-induced pH increase by excess RU28318. Lower panel: dose–response curve for cortisol induction of rapid nongenomic effect in the presence of carbenoxolone. Adapted from [5].

1.3. Acute vascular effects of aldosterone: in vivo studies

More recently, Romagni et al. have documented the effects on forearm blood flow, as a measure of vascular resistance, in patients receiving a brief infusion of a modest level of aldosterone into one brachial artery [19]. Compared with the contralateral forearm, flow decreased significantly by 4 min after the start of infusion, to reach a nadir at 12 min and returned to baseline over a similar period. Though these studies do not distinguish primary vascular smooth muscle from endothelial effects, they constitute persuasive data for significant rapid vasoconstrictor responses to plasma aldosterone levels measured as still within the normal physiologic range.

1.4. Acute cardiac effects of aldosterone: in vitro studies

In a series of studies from the Mihailidou laboratory, aldosterone has been shown to have rapid (<15 min) effects on both the $Na^+/K^+/2Cl^-$ cotransporter and Na^+/K^+ pump activity in isolated rabbit cardiomyocytes [6,7]. When cardiomyocytes are exposed to 10 nM aldosterone, pump current increases ≥10-fold within 15 min. This reflects a direct effect on $Na^+/K^+/2Cl^-$ cotransport, leading to an increase in intracellular Na^+ concentration and thus a substrate-driven increase in pump activity. When the cotransporter is blocked by bumetanide, aldosterone at 10 nM produces a reduction in pump activity, to levels 60–70% control. The aldosterone effect on the cotransporter is not seen after chronic (7 days) aldosterone infusion, whereas the direct inhibitory effect on the pump persists—though

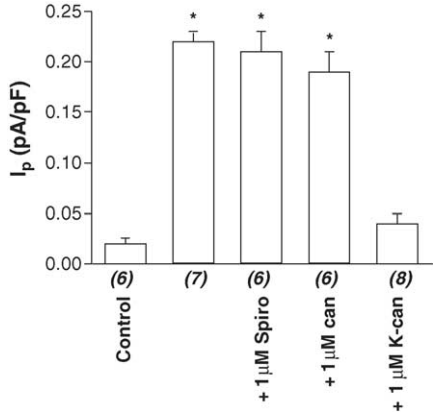

Fig. 4. Rapid nongenomic effect of aldosterone on pump current in isolated cardiomyocytes. The closed ring MR antagonists spironolactone (spiro) and canrenone (can) do not significantly block the aldosterone effect; the open E-ring, water soluble MR antagonist potassium canrenoate (K-can) does. $^{*}P < 0.05$.

of course after 7 days there is no way that nongenomic and possible genomic effects can be distinguished.

That these effects appear to be mediated via classical MR, like those previously described for VSMC, is supported by the data shown in Fig. 4. The acute effect of aldosterone 10 nM on cotransporter activity is not blocked by spironolactone or canrenone at 100-fold excess. In contrast, potassium canrenoate, the open E-ring, water soluble MR antagonist blocks the aldosterone effect, just as RU28318 did in VSMC. This effect of aldosterone is mediated via PKCε at both cotransporter and pump level. Staurosporine, bisindolylamide and a PKCε specific inhibitory peptide all blocked the aldosterone-induced increase in cotransporter activity; in addition, an agonist PKCε peptide administered via the broad-tipped pipet mimicked aldosterone action, whereas PKCα, PKCδ and a scrambled PKCε agonist peptides did not. Identical results, in terms of PKC, were obtained for the pump itself when Na^+ influx was blocked by bumetanide in acute studies, with PKCε mimicking the acute inhibitory effect of aldosterone.

Although as noted above it is not possible to definitively distinguish nongenomic and genomic effects of aldosterone after 7 days of steroid administration, the data in Fig. 5

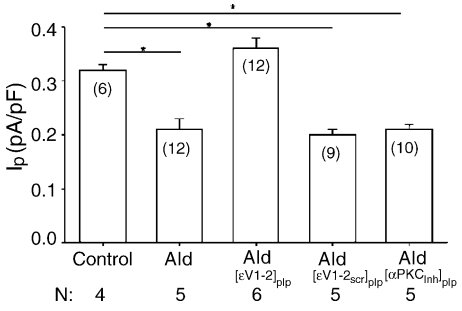

Fig. 5. Chronic effect of aldosterone on pump current in isolated cardiomyocytes, reversal by PKCε inhibitory peptide $[\varepsilon V1\text{-}2]_{pip}$, but not by a scrambled PKCε inhibitory peptide $[\varepsilon V1\text{-}2_{scr}]_{pip}$, or by a PKC$\alpha$ inhibitory peptide $[\alpha PKC_{int}]_{pip}$. $^{*}P < 0.05$. Adapted from [7].

suggest that the aldosterone effect on the pump may be nongenomic even after 7 days. When rabbits are infused for 7 days with aldosterone at a dose to give plasma levels two- to three-fold normal, myocytes show significantly reduced pump activity compared with myocytes from vehicle-infused animals. When, however, the PKCε inhibitory peptide $[\varepsilon V1\text{-}2]_{pip}$ is infused via the pipet the chronically induced aldosterone effect on pump current is fully reversed within 15 min, this effect is not reproduced by PKCε scrambled or PKCα inhibitory peptides. The effect of 7 days aldosterone has not only persisted for 4 h, the average time from animal sacrifice to recording, but can be promptly reversed by PKCε inhibition, just as the acute effects of aldosterone on both cotransporter and pump can be blocked by coadministration of PKCε inhibitor.

While these studies cannot exclude a genomic component of the aldosterone effect over 7 days, they show that the acute effect on the pump may also be chronic, that it persists over 4 h of myocyte isolation in steroid-free media and that it can be acutely reversed by PKCε inhibition. Thus, nongenomic effects may not be necessarily evanescent, but may in addition be chronic and persistent. It is also worth noting that this chronic effect of aldosterone on pump current can be blocked in vivo by spironolactone [20]; why the closed E-ring MR antagonists (spironolactone, canrenone) but not open E-ring blockers (RU28318, potassium canrenoate) are ineffective in the acute experiments remains to be established.

In the VMSC studies from Marusic's laboratory [5] cortisol became an agonist when carbenoxolone was added to block 11βHSD2, and thus the conversion of cortisol to cortisone and the generation of NADH. Cardiomyocytes normally express very low levels of 11βHSD2 [20], so that their MR are normally overwhelmingly occupied by cortisol, in tonic inhibitory mode; when 11βHSD2 transgenic mice are made to express the enzyme specifically in cardiomyocytes, they develop dilated cardiomyopathy and heart failure, presumably reflecting inappropriate occupation and activation of cardiomyocyte MR by aldosterone [20]. Progression of heart failure in these 11βHSD2 transgenic mice is partially ameliorated by eplerenone from 2 months of age [20] and the phenotype is abolished by cardiomyocyte selective MR knockout in the 11βHSD2 transgenic mice [21].

Whether tissues express 11βHSD2 (e.g., VSMC) or not (cardiomyocytes) the pathophysiologic circumstances of intracellular redox change are likely to be the generation of reactive oxygen species (ROS). In very recent preliminary studies [22], we have mimicked ROS-induced changes in intracellular redox status in rabbit cardiomyocytes by instilling oxidized glutathione (GSSG, 5 mM) into the cells via the broad tipped pipet. GSSG infused alone has no effect on pump current, nor does cortisol 100 nM. When cortisol 100 nM is infused with aldosterone 10 nM, it blocks the aldosterone effect stoichometrically, down to ∼10% of maximum. When, however, cortisol is infused with GSSG, to mimic 11βHSD2 blockade in VSMC or ROS

generation more generally, the glucocorticoid switches from mineralocorticoid receptor antagonist to agonist, mimicking rather than blocking the aldosterone effect.

This demonstration of the bivalent nature of the cortisol–MR complex may thus begin to provide an insight into the mechanism of action of an always-occupied (by glucocorticoid) receptor. Glucocorticoid receptors (GR) have much lower affinity for cortisol (and corticosterone) than MR, and thus are more or less occupied and activated over the normal diurnal/stress range of circulating glucocorticoids. MR, on the other hand, are essentially always occupied by glucocorticoids in tissues, particularly those lacking 11βHSD2 to debulk intracellular glucocorticoid levels. Cardiomyocyte MR – or those in most neurons – thus cannot respond to changes in ambient glucocorticoids; what may determine their activation, in contrast, is the metabolic state of the cell, as reflected in its redox status. We are used to thinking of glucocorticoids as stress hormones acting via GR; their role as constitutive occupants, but metabolically determined activators, of MR in response to cellular stress awaits exploration.

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