Effects of Aldosterone and Mineralocorticoid Receptor Blockade on Intracellular Electrolytes

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Abstract. Genomic mechanisms of mineralocorticoid action have been increasingly elucidated over the past four decades. In renal epithelia, the main effect is an increase in sodium transport through activation and de novo synthesis of epithelial sodium channels. This leads to increased concentrations of intracellular sodium activating sodium-potassium-ATPase molecules mainly at the basolateral membrane which extrude sodium back into the blood stream. In contrast, rapid steroid actions have been widely recognized only recently. The present article summarizes both traditional and rapid effects of mineralocorticoid hormones on intracellular electrolytes, e.g. free intracellular calcium in vascular smooth muscle cells as determined by fura 2 spectrofluorometry in single cultured cells from rat aorta. Latter effects are almost immediate, reach a plateau after only 3 to 5 minutes and are characterized by high specificity for mineralocorticoids versus glucocorticoids. The effect of aldosterone is blocked by neomycin and short-term treatment with phorbol esters but augmented by staurosporine, indicating an involvement of phospholipase C and protein kinase C. The Ca$^{2+}$ effect appears to involve the release of intracellular Ca$^{2+}$, as shown by the inhibitory effect of thapsigargin. This mechanism operates at physiological subnanomolar aldosterone concentrations and appears to result in rapid fine tuning of cardiovascular responsivity.

As a landmark feature of these rapid effects, insensitivity to classic antimineralocorticosteroids, e.g. spironolactone or canrenone has been found in the majority of observations. In an integrated view, mineralocorticoids seem to mainly effect intracellular electrolytes genomically to induce transepithelial transport, and induce nongenomically mediated alterations of cell function (e.g. vasoconstriction) by rapid effects on intracellular electrolytes such as free intracellular calcium.

Key Words. mineralocorticoids, intracellular electrolytes, rapid effects, second messengers

Introduction

It has been over 40 years since Crabbé showed that aldosterone enhances electrogenic transepithelial sodium (Na) transport in the toad urinary bladder [1]. Edelman et al. [2] demonstrated that this action was mediated through increased RNA and protein synthesis, and thus relates to a classical genomic effect involving intracellular, traditional steroid receptors. It took another 2 decades to identify and finally clone those mineralocorticoid receptors belonging to the superfamily of intracellular steroid receptors [3].

One of the targets of the early research activities was the search for the molecule that permitted Na entry across the apical membrane of the collecting duct, which was identified as the epithelial sodium channel (ENaC). The discovery, by Canessa et al. [4] that this epithelial Na channel (ENaC) comprised three homologous subunits was one of the major breakthroughs of this long search, which finally led to the cloning of this channel by Rossier’s group [5]. In addition, the discovery of ENaC permitted the identification of the molecular correlate of Liddle’s syndrome, a severe form of hereditary, familial hypertension in which the channel is constitutionally activated even in the absence of circulating hormone [6]. Other genetic diseases related to this channel include glucocorticoid-remediable hypertension, which produces an excessive amount of aldosterone [7]; apparent mineralocorticoid excess, which involves defects in the enzyme 11-beta-hydroxysteroid dehydrogenase–type II and a failure to inactivate cortisol in the principle cell [8]; and a recently identified activating mutation in the mineralocorticoid receptor [9].

Over the past two decades, this genomic part of the theory on mineralocorticoid action has been supplemented by novel observations on instant effects of mineralocorticoids which become visible within seconds to few minutes. These effects are by far too fast to involve the genomic machinery and therefore called nongenomic effects. They are resistant to inhibitors of transcription (e.g. actinomycin D) or protein synthesis (e.g. cycloheximide), and—in the case of mineralocorticoids—expose a characteristic pharmacology: they are (few exceptions may exist) resistant to classical antimineralocorticoids. Their physiological function is not...
yet as clear as that of the genomic effects, but
seems to include instant cardiovascular changes,
e.g. increases in vascular resistance, which might
qualify aldosterone as acutely acting cardiovascu-
lar hormone (for review see 10).

This review comprises data on both the traditional
genomic as well as those novel nongenomic
effects of aldosterone, and the impact of antimin-
eralocorticoid drugs on intracellular electrolytes.

Classical Action of Aldosterone
in Tight Epithelia

It has been known for several years that one of
the gene products induced by aldosterone is ENaC.
Though it is certainly not the only gene that is
directly regulated by aldosterone[11], but it seems
to be of major importance.

To understand this effect of aldosterone, we
need to briefly look at serum and glucocorticoid in-
ducible kinase 1 (SGK1), a very early gene that is
induced by aldosterone, which is also upregulated
by glucocorticoids.

SGK1 seems to represent an important conver-
gence point for various regulators of Na\(^+\) trans-
port (for review see 12). It is under both ex-
pression and functional control: protein levels are
controlled through effects on its gene transcription,
while its activity is clearly regulated by phospha-
tidylinositol-3-kinase (PI3K) activity. Al-
dosterone is the most effective regulator of SGK1
protein expression in ion transporting epithelia;
insulin and other known activators of the PI3K
have major impact on its activity. Activated SGK1
regulates a variety of ion transporters, includ-
ing the epithelial sodium channel (ENaC) [13].
The apical targeting of ENaC seems to depend on
Nedd4-2 phosphorylated by SGK1 [14]. This effect
of SGK1 requires close physical contacts of Nedd4-
2 with both SGK1 and ENaC.

The SGK family represents an ancient arm of
the serine-threonine kinase family, present in all
eukaryotes that have been examined, including
yeast.

Some of the important features of ENaC regula-
tion in the collecting duct include a large increase
in activity after NaCl restriction or mineralocorti-
coid administration to the intact animal [15] and
an increase in the mRNA levels of alpha-ENaC
after aldosterone infusion [16,17].

Long-term effects of mineralocorticoids on blood
pressure and on electrolyte and water balances are
believed to result from genomic effects in the distal
tubule of the kidneys [18] through the related in-
crease of ENaC activity/expression. It is generally
assumed that these effects are mediated by cyto-
plasmic type-I receptors (mineralocorticoid recep-
tors (glucocorticoid receptors), but bind mineralo-
corticoids and glucocorticoids equally well. As with
other steroids, the receptor-ligand complex acts as
a transcription factor and initiates the transcrip-
tion of specific mRNA encoding for aldosterone-
induced proteins [22]. Na\(^+\) reabsorption across
aldosterone target epithelia utilizes a two-step
mechanism. Na\(^+\) ions enter the cells driven by an
electrochemical gradient through the apically con-
centrated epithelial Na\(^+\) channel (ENaC); they are
then actively excreted into the extracellular space
by the basolaterally concentrated Na\(^+\) pump (Na\(^+\),
K\(^+\)-ATPase).

The response to aldosterone has been exten-
sively studied in amphibian model epithelia, such
as the urinary bladder of Bufo marinus and A6
cells derived from the distal nephron of Xenopus
laevis [23,24]. The increase in transepithelial Na\(^+\)
transport begins after a delay of approximately
30–60 min. During an initial phase which reaches
a maximum 2–4 h after hormone addition, pre-
existing Na\(^+\) channels and Na\(^+\) pumps seem to
be recruited and activated. A second phase start-
ing within 3–6 h after aldosterone application, is
characterized by an increase in the number of Na\(^+\)
channels and Na\(^+\) pumps. These effects are only
seen in epithelia expressing the classic intracellu-
lar mineralocorticoid receptors and are fully sen-
sitive to inhibitors of transcription or translation,
thus indicating the involvement of the genomic
machinery.

Specificity for mineralocorticoids over gluco-
corticoids which bind to mineralocorticoid recep-
tors equally well is—among other mechanisms
discussed [25]—conferred in target epithelia by
the presence of the 11ß-hydroxysteroid dehydro-
genase type 2. This enzyme degrades glucocorti-
coids into inactive compounds and thereby pre-
vents the mineralocorticoid receptors from being
activated by glucocorticoids, a theory which seems
to be largely, but not entirely sufficient to explain
the clinical differences of gluco- vs mineralocorti-
coids on sodium and potassium balance.

The epithelial Na\(^+\) channel is the limiting bot-
tleneck for the transepidermal transport of sodium,
if the Na\(^+\) pump maintains an adequate driving
force. Three subunits of this channel have been
cloned (see above). In terms of the aldosterone ef-
fect on this channel, it was [26] shown that the api-
cal permeability of toad bladders treated with al-
dosterone was increased by at least two different,
subsequent mechanisms. The early effect on api-
cal Na\(^+\) conductance observed in bladders within 3
hours of incubation with aldosterone appeared to
be due to a reversible activation of preexisting Na\(^+\)
channels with increased open probability. After
stimulation followed by aldosterone withdrawal,
the open probability of the channels slowly recedes
to baseline [27].