Early aldosterone action: toward filling the gap between transcription and transport

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Verrey, François. Early aldosterone action: toward filling the gap between transcription and transport. *Am. J. Physiol.* 277 (*Renal Physiol.* 46): F319–F327, 1999.—The mineralocorticoid hormone aldosterone stimulates transcellular Na^+ reabsorption across target epithelia after a lag period of 20 to 60 min by first activating preexisting channels (epithelial sodium channels, ENaC) and pumps (Na-K-ATPase) and, subsequently, increasing the overall transport capacity of the cells. Both these early regulatory and late anabolic-type actions depend on the transcriptional regulation exerted by hormone-activated mineralocorticoid and/or glucocorticoid receptors (MR and/or GR). Starting at the transcriptional side of the aldosterone action, recent studies have identified the small G protein K-Ras2 and the kinase sgk as the first early aldosterone-induced gene products potentially regulating Na^+ transport. At the level of the Na^+ transport effectors, much knowledge about ENaC and Na-K-ATPase structure-function relationship and regulation has accumulated. However, the regulatory pathway(s) that link the transcriptional action of aldosterone to these Na^+ transport proteins is still to a large extent unknown. The available data suggest that the early regulatory action of aldosterone is pleiotropic, similarly to the late anabolic-type action. The early Na^+ transport stimulation would be mediated by the rapid induction of gene products belonging to the regulatory network that integrates the inputs of diverse pathways and finally controls the function of the Na^+ transport machinery.

epithelial sodium ion transport; K-Ras; glucocorticoid receptor; mineralocorticoid receptor; messenger ribonucleic acid stability; sgk

CLASSIC ALDOSTERONE target epithelia are electrically tight monolayers lining the distal part of excretory organs such as the kidney distal nephron (distal tubule to papilla), distal (rectal) colon, sweat glands, and salivary glands. Many of the concepts applicable to these Na^+ (re)absorbing epithelia were originally formulated on the basis of experimental work performed with amphibian model epithelia such as the toad bladder and the epithelia formed by *Xenopus laevis* kidney A6 cells. The actions of aldosterone on other tissues and organs, such as heart, vascular system, and central nervous system, appear to play an important role in the systemic response to aldosterone as well. These have as yet not been studied as extensively and are not discussed in this short review.

The fact that aldosterone requires ongoing transcription in its target epithelia to exert its physiological action has been known for decades, and the question of transcriptionally induced mediators (aldosterone-induced proteins, AIP) mediating this action has attracted much attention. The first approach to this question was a "candidate approach," and the first candidates to be considered were the major effectors of transcellular Na^+ transport, which are the apical epithelial Na^+ channel (ENaC) and the basolateral Na^+ pump (Na-K-ATPase). These effectors have been shown to be regulated in their number, at least in the long term, by aldosterone (see below). This late and very late effect on target epithelia can be viewed as an anabolic action leading to a state of cellular differentiation that allows a higher transport activity. However, upon treating target epithelia with aldosterone, the beginning of the physiological response precedes the accumulation of effectors, indicating that there must be an earlier, "regulatory" phase of aldosterone action that precedes the anabolic phase. Thus it has been postulated that a regulatory protein(s) would be induced/repressed by aldosterone, which activates directly or indirectly the function of preexisting effectors, namely, ENaC and Na-K-ATPase.

There are two different ways of approaching the question of aldosterone-induced regulatory proteins. The first consists of starting from the effector side and following the pathway backward by analyzing the

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regulation at the level of the function of ENaC and/or the Na-K-ATPase, identifying or postulating regulators of these transport effectors, and subsequently testing whether they are induced by aldosterone.

The second, forward approach to the question of the mechanism of aldosterone action starts at the other side of the gap, that is, at the level of the transcriptional event. This approach consists of identifying aldosterone-regulated gene products and subsequently asking the question of whether these regulated gene products are involved in the physiological action of the hormone. New powerful techniques to analyze differential gene expression are now becoming available (11, 68) such that the results obtained as yet by this second approach and discussed below only represent a beginning.

The main aim of this short review is to discuss the recent data that are beginning to fill the void of knowledge of what lies between the transcriptional events induced by aldosterone and the early activation of Na^+ transport effectors (see the model for mechanism of aldosterone action shown in Fig. 1). Some aspects concerning the specificity of the hormone action, new mechanisms of nuclear receptor action, and the "nongenomic" pathway for aldosterone action are also briefly discussed. For other aspects of the aldosterone action, the readers are referred to other recent reviews (7, 15, 20, 35, 60–62, 65).

ALDOSTERONE AND THE QUESTION OF MINERALOCORTICOID SPECIFICITY

Aldosterone exerts what is called a mineralocorticoid action, meaning that it stimulates salt retention and thus also impacts on extracellular volume and blood pressure control. This action at the systemic level is clearly different from that of glucocorticoid hormones. Despite this clear difference, the question of specificity is quite complex downstream of the hormones.

Indeed, the classic corticosteroid nuclear receptors (high- and low-affinity receptors called MR and GR) are not restricted in their specificity to aldosterone and cortisol/corticosterone, respectively, but bind both hormones similarly. Conversely, MR and GR can each exert typical gluco- and mineralcoricosteroid actions, in certain conditions. Furthermore, in some cells, it appears that the MR essentially plays a role of a high-affinity receptor for glucocorticoids, extending the range of glucocorticoid action (1, 45).

The major factor providing hormone specificity in aldosterone target cells is the prereceptor barrier represented by the 11β-hydroxysteroid dehydrogenase type 2, which transforms the corticosteroids to the corresponding inactive 11-oxocorticosteroids (see bibliographies of Refs. 35, 62, and 65 for pertinent references). It is noteworthy that this barrier for glucocorticoids opens

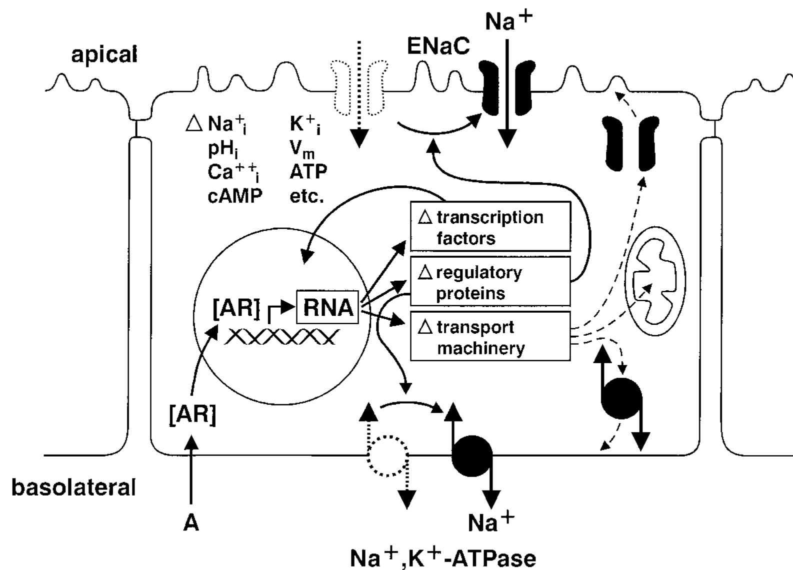

Fig. 1. Model for the mechanism of aldosterone action in epithelial target cell. mRNA and protein levels of early aldosterone-induced/repressed gene products are modified starting during the physiological lag phase (20–60 min). K-Ras and sgk are the first early aldosterone-induced regulatory proteins identified as yet (10, 29, 51). It is expected that, together with other induced/repressed regulatory proteins, they stimulate the activity of ENaC and Na-K-ATPase during the early phase of aldosterone action (up to 2–3 h after hormone addition). Aldosterone (A) has also been shown in A6 epithelia to rapidly activate or repress the expression of transcription factors involved in differentiation or proliferation, respectively, thus tuning genome programming toward differentiation. Interestingly, the decrease in c-*myc*, c-*fos*, and c-*jun* mRNAs was shown to be independent of transcription (51). In a cell-type-specific mode and in coordination with other hormones and Na^+ load, aldosterone promotes the synthesis of ENaC and Na-K-ATPase and of other elements of the Na^+ transport machinery. Some of these latter anabolic effects are initiated early (i.e., induction of Na-K-ATPase and LAT1 transcription in A6 cells; see Refs. 5 and 50) but belong functionally to the late phase of aldosterone action which starts ~3 h after hormone addition (stippled arrows) (5). Several cellular parameters are indicated [e.g., intracellular Na^+, K^+, transmembrane potential (V_m, etc.] that can influence the response to aldosterone. These same parameters are potentially modified (Δ) by the action of aldosterone and mediate indirect effects. AR, aldosterone-receptor complex.

the way for aldosterone to bind not only to the high-affinity MR but also to the more numerous, but low-affinity, GR. In addition to the prominent specificity-conferring effect of 11β-hydroxysteroid dehydrogenase, other more subtile levels of specificity are provided at other cellular levels, for instance, by the expression of corticosteroid hormone transporting membrane proteins such as organic anion transporters (OATP), multidrug resistance protein (MDR), and possibly others (8, 58) and by differences in the action of MR vs. GR at the level of certain types of response elements on target genes (26, 27, 38, 44, 56). The physiological implications of these differences are not yet clear. Furthermore, the activity of the receptors depends also on the liganded natural or synthetic hormones that differentially activate MR and GR by behaving as (partial) agonist and/or antagonists (14, 45).

Another important point concerning the specificity of the action of aldosterone is the fact that this steroid hormone works in concert with a large number of other hormones and factors also implicated in the control of salt and volume homeostasis and blood pressure via a complex network of regulatory cascades. Thus permissive conditions are required to enable aldosterone to fully produce its mineralocorticosteroid action in target cells. Indeed, at many levels of the cellular cascade, from the hormone binding, via the transcription, and down to the regulation of the function of the effectors, other regulatory inputs can limit/prevent the action of aldosterone (produce an escape) or, alternatively, reinforce it by acting synergistically.

In conclusion, the term "mineralocorticoid" has unfortunately not an equivalent meaning at the level of the physiological action, the receptors, and the hormones. Indeed, mineralocorticoid action in the sense of salt-retaining action can be mediated by glucocorticosteroids as well (see the case of apparent mineralocorticosteroid excess) and is also not uniquely mediated via MR, since it can be mediated via GR, to a large extent (25, 48). Furthermore, maximal Na^+ transport activation requires the simultaneous activation of both types of receptors (MR and GR), as shown in the toad bladder and assumed for mammalian target epithelia (17).

NEW PLAYERS AT THE LEVEL OF THE TRANSCRIPTIONAL ACTION OF NUCLEAR RECEPTORS

The concept of how nuclear receptors act on transcription has been refined during the past few years. More players are emerging that are involved in the transactivation and/or transrepression exerted by nuclear receptors on regulated genes with classic hormone binding elements in their regulatory sequences. Coactivators and/or corepressors have been identified which bind to the activated receptors and act at the level of the chromatine structure by acetylating/deacetylating histones and at the level of the transcription initiation complex by bridging the nuclear receptors to the general transcription machinery and other transcription factors (see bibliography of Ref. 65 for pertinent references).

Another important point, the physiological relevance of which has been recently demonstrated by a transgene experiment performed by the group of Schütz (41), is the fact that nuclear receptors play crucial roles independent of their capability to bind specific DNA regulatory sequences (41). Specifically, it was shown that a GR rendered unable to bind DNA, by means of a mutation impairing homodimerization, is sufficient to allow survival of mice, in contrast to the absence of GR, which is lethal. Correspondingly, it has been shown that some genes devoid of GREs (corticosteroid hormone regulatory elements, i.e., MR and GR binding sequences) can be activated or repressed by liganded nuclear receptors via protein-protein interactions with other transcription factors (32, 33, 55). The multiple types of interactions that can be made by activated nuclear receptors enables the integration of multiple signals such that the action of aldosterone at the level of the transcription of target genes has to be viewed as one element within a complex network controlling their regulation. It can be concluded that the extent of regulation of a target gene by a given concentration of aldosterone depends on many gene- and cell-specific parameters as well as on the physiological context.

"NONNUCLEAR" ALDOSTERONE RECEPTORS AND THE MINERALOCORTICOID ACTION

Over the past years, much data has accumulated showing that, in addition to the classic nuclear receptors which are ligand-activated transcription factors, there are also other receptors for aldosterone that mediate rapid actions, independent of transcription and translation. A rapid increase in inositol trisphosphate, Ca^{2+}, and/or protein kinase C activity as well as an activation of Na^+/H^+ exchange and K^+ channels have been observed in several cell types, in particular in vascular smooth muscle and endothelial cells (60, 66). However, the biochemical nature of the receptors mediating these actions remains to be established. The physiological role of the rapid nongenomic aldosterone action is not yet clear. Recently, the group of Wehling (67) has shown in humans that intravenous injection of aldosterone has a rapid, transient effect on hemodynamic parameters.

The presence of nonnuclear aldosterone receptors is also postulated in some tight epithelia expressing classic corticosteroid receptors as well, since rapid nongenomic effects of aldosterone such as the activation of Na^+/H^+ exchange and an increase in intracellular Ca^{2+} have been observed in such cells (12, 18, 66). However, verifying and understanding the potential role of nongenomic aldosterone actions on the regulatory network that controls Na^+ transport in epithelial target cells will require further investigations.

ALDOSTERONE-INDUCED PROTEINS INVOLVED IN LATE ANABOLIC ACTION

The fact that long-term aldosterone increases the transport capacity of target epithelia has been men-

tioned above. Here, I want to briefly mention the "constitutive" gene products known to be induced by aldosterone and thereby to participate in the late aldosterone action. The Na-K-ATPase has been shown to be induced rapidly at the transcriptional level by aldosterone in A6 epithelia (64). However, since the pool of the corresponding mRNAs and proteins are large, also in the absence of aldosterone, and since their half-lives are long, changes at the level of mRNA and protein synthesis are delayed (6, 36, 64). This results in a significant increase in active cell surface pumps being detected only 5 h after aldosterone addition (5, 6). Another point is that the accumulation of Na-K-ATPase mRNAs and functional pumps also depends on other, coregulatory factors, such as the Na^+ concentration and triiodothyronine (62). Finally, there are clearly also target cell-specific factors that influence this regulation. At the level of the kidney collecting duct, for instance, it appears that only the α-subunit mRNA of the Na-K-ATPase is under aldosterone control, whereas it is not regulated at the level of the distal colon (13).

Similarly, the subunits of ENaC are differentially regulated in kidney and colon. For instance, in the kidney cortical collecting duct, aldosterone increases ENaC α-subunit mRNA without affecting the mRNAs encoding the two other subunits. In contrast, in colon, β- and γ-subunit mRNAs are upregulated by aldosterone or dexamethasone, whereas the α-subunit mRNA is expressed constitutively (2, 13, 42). Similar conclusions were obtained concerning the regulation of ENaC subunits at the protein level using immunohistochemistry (42). In the A6 cell line, apart from late increase in the abundance of all three subunit mRNAs (31, 50), aldosterone also specifically increases the rate of α-subunit protein synthesis already 60 min after the start of the treatment. A few hours later, the rate of synthesis of the β-subunit, but not that of the γ-subunit, is increased as well (31). The fact that ENaC α-subunit synthesis is increased before the increase in mRNA indicates that aldosterone, in this case, also acts at the posttranscriptional level of the expression of a Na^+ transport effector protein. This translational effect takes place during the early phase of aldosterone action. Despite this early timing, it is not yet clear whether this effect plays a functional role during the early phase of aldosterone action, since it has not been measured at the level of functional surface channels.

Other gene products involved in Na^+ transport across aldosterone target cells have been shown to be regulated by aldosterone as well. Since the extrusion of K^+ is a prerequisite for Na^+ transport (because of the exchange function of the Na-K-ATPase) and since aldosterone also stimulates apical K^+ secretion in the collecting duct, K^+ channels are also considered as candidates for regulation by aldosterone. Indeed, the mRNAs of ROMK2 and ROMK3, which are involved in K^+ secretion by principal cells of the kidney collecting duct, have been shown to be induced by long-term aldosterone using competitive PCR (4). The kidney distal convoluted tubule expresses MR and thus represents a target for aldosterone action, similar to the connecting tubule and the collecting duct. Very recently, the apical Na^+-Cl^- cotransporter, which is the main apical Na^+ transport protein in this segment, has been shown to be strongly induced by aldosterone in the long term (34). A protein from the same family as the γ-subunit of the Na-K-ATPase, CHIF, has been identified as strongly regulated by long-term aldosterone in distal colon (not in kidney). Oocyte expression experiments and its regulatory behavior indicate that it might exert a function in the control of K^+ transport (3).

Other gene products that have been shown at the mRNA level to be induced by aldosterone and which can be considered to play a role for the late anabolic response are mitochondrial enzymes (identified in rat colon) involved in the energy metabolism (40) and the amino acid transport protein LAT1 (30). The transcript of this latter gene product has been identified as early induced mRNA by differential display PCR in A6 cells (Ref. 50; also, see below) and the LAT1 protein subsequently shown to require association with a glycoprotein for surface expression (30). It is upregulated early, but only twofold, in the course of the response to aldosterone and could play a role by promoting the uptake of amino acids used for energy metabolism, protein synthesis, and/or volume regulation.

The separation of early and late effects of aldosterone appears slightly artificial, because several gene products mentioned above might initially be regulated by aldosterone directly at the transcriptional level early in the course of its action, as mentioned above for the Na-K-ATPase in A6 epithelia. In contrast, the amino acid transport protein LAT1 was mentioned in the context of the late response despite an early increase at the mRNA level. This is justified by the fact that LAT1 does not appear to have a regulatory role but rather appears to be a constitutive element which potentially exerts a supportive role for the anabolic action of aldosterone. Thus the temporal division of aldosterone-induced proteins in early and late ones might not always reflect regulatory versus anabolic functions.

ALDOSTERONE-INDUCED PROTEINS POTENTIALLY INVOLVED IN EARLY SODIUM ION TRANSPORT REGULATION

The first early aldosterone-induced mRNAs were identified in A6 epithelia. Using differential display PCR to compare cDNA fragments generated from RNA of control and aldosterone-treated (40 and 60 min) epithelia, Spindler and colleagues (50) showed that only a small proportion of the RNAs ($<0.5\%$) was significantly regulated within the lag period of aldosterone action and hence potentially involved in the mediation of its early effect. For two of the cloned cDNA fragments corresponding to ASURs ("adrenal steroid upregulated RNAs"), cDNAs encoding the entire protein coding sequences were isolated and further characterized (see below), whereas the two other ASURs did not appear to be typical mRNAs. From the two characterized mRNAs, ASUR4 was shown to encode a novel amino acid transporter that might play a role in the anabolic action of aldosterone, despite its early induc-

tion (see above), whereas ASUR5 encodes the *Xenopus* ortholog of mammalian K-Ras2 (*X*K-Ras2). Northern blot and PCR experiments indicated that both the A and B splice variants of *X*K-Ras2 are similarly expressed and regulated by aldosterone (Spindler and Verrey, unpublished results). The K-*ras* mRNA has been recently shown to be regulated also in vivo at the level of the *Xenopus* kidney by aldosterone (2.5 h) (51). Furthermore, the biosynthesis of Ras proteins was shown to be increased approximately sixfold in A6 epithelia 2.5 h after aldosterone addition (51).

Experiments to test whether an increase in K-Ras2 activity could act on the function of epithelial Na^+ channels were performed in the *Xenopus* oocytes expression system (29). *X*K-Ras2 rendered constitutively active by the G12V mutation was shown to have a dual action on coexpressed ENaC. On the one hand, it induced oocyte maturation, thereby promoting endocytosis of surface transporters, including endogenous Na^+ pumps and exogenously expressed ENaCs. On the other hand and in accordance with the hypothesis that K-Ras2 exerts a stimulatory action on Na^+ transport, the activity of the surface expressed channels was increased fourfold relative to that of the channels expressed at the surface of control oocytes (Fig. 2). However, an increase in K-Ras expression is expected to activate downstream effectors, and possibly Na^+ channels, only if it is itself activated by upstream regulators. This indicates that K-Ras is not the single key regulator in the mediation of Na^+ transport stimulation by aldosterone but that other regulatory proteins must also play an important role.

The serum and glucocorticoid-induced kinase sgk has been shown very recently by Chen and colleagues (10) to be strongly and very rapidly regulated in A6 epithelia by aldosterone and dexamethasone. Furthermore, in situ hybridization studies have shown that this protein is expressed in the mouse kidney with a pattern corresponding to collecting duct and glomeruli. Only in the collecting duct was the signal increased upon treatment of the animals with aldosterone (4 h). Coexpression experiments with ENaC in *Xenopus* oocytes have shown that the presence of sgk strongly increases the ENaC-mediated Na^+ current (Fig. 2), in contrast to an absence of effect on ROMK2-mediated K^+ current. The target(s) for phosphorylation by sgk leading to an increase in ENaC-mediated Na^+ current has not been identified as yet, and it has not been excluded that ENaC is this target itself. This would be compatible with the results of a recent study suggesting that the phosphorylation of ENaC β- and γ-subunits expressed in MDCK cells is increased after an aldosterone treatment (49). In any case, the stimulatory effect of sgk is not general, and the fact that the function of ROMK2 is not increased by sgk is particularly interesting in view of the fact that this K^+ channel is expressed on the same apical membrane as ENaC in collecting duct principal cells, where it is thought to mediate, at least in part, the K^+ secretion (19). The lack of ROMK regulation by sgk is compatible with the hypothesis that sgk plays an important role in the control of Na^+ and K^+ transport by these cells, since K^+ secretion is coupled to Na^+ reabsorption due to the impact of the Na^+ conductance on the membrane potential. As mentioned above, aldosterone appears to control the number of K^+ channels in the long term (4).

It is possible that the kinase sgk has a number of different upstream regulators and downstream effectors, as it is known to be the case for the small G protein K-Ras2, the other early aldosterone-induced regulatory protein identified as yet. The fact that aldosterone rapidly induces more then a single regulatory protein and that these proteins potentially have a number of upstream regulators and downstream targets indicates that the regulatory action of aldosterone on Na^+ transport has a pleiotropic character and might impact differentially in different target cells, even if a single aldosterone-induced protein might play a key (rate-limiting) role for Na^+ transport regulation in some situations.

This view is also supported by a number of observations indicating that aldosterone acts, at least later in the course of its action, on proteins belonging to other signaling cascades which regulate ENaC function and Na^+ transport. For instance, aldosterone has been shown to potentiate antidiuretic hormone-induced

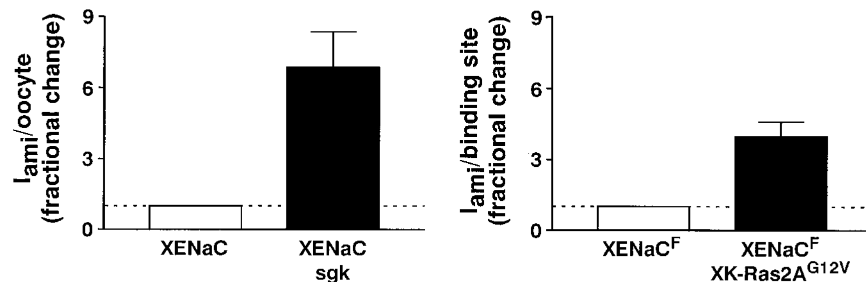

Fig. 2. Aldosterone-induced proteins sgk and K-Ras2A (G12V mutation) increase the function of ENaC (*Xenopus laevis*, α, β, γ-subunits; "XENaC") coexpressed in *X. laevis* oocytes. In presence of coexpressed sgk, amiloride-sensitive current per oocyte (at a membrane potential of −100 mV; I_{ami}/oocyte; *left*) was significantly higher than in its absence (mean control I_{ami} = −1.52 μA/oocyte). In contrast, in presence of *X*K-Ras^{G12V}, mean I_{ami} per oocyte was not significantly higher than in controls (−4.6 ± 0.6 and −4.3 ± 0.6 μA/oocyte, respectively). However, in presence of *X*K-Ras2A^{G12V}, the number of surface channels measured by anti-FLAG antibody binding was reduced (using FLAG epitope-tagged ENaC^F), such that the calculated mean activity per surface-expressed channel (*right*) was correspondingly higher (mean control I_{ami}/binding site = 15 ± 2 μA/fmol; 3 binding sites/tetrameric channel). Data are from Refs. 10 and 29.

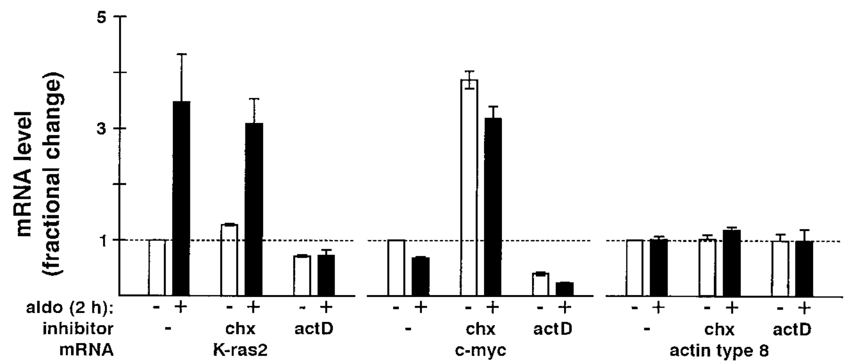

Fig. 3. Examples of differential early regulation of mRNAs by aldosterone in A6 epithelia. The gene encoding *X*K-Ras2 (ASUR5) as well as those of the other ASURs (50), the kinase sgk (10), and the transcription factor Fra-2 (51) are directly, transcriptionally regulated by aldosterone (aldo), since the corresponding mRNAs are rapidly upregulated by a transcription-dependent mechanism [inhibited by actinomycin D (actD)], but independent of ongoing translation [not inhibited by cycloheximide (chx)]. In contrast, c-*myc*, as well as c-*jun*, and c-*fos* mRNAs are downregulated independent of transcription and translation. Inhibition of translation per se increased the level of their mRNAs. Cytoskeletal actin (type 8) mRNA is not affected by aldosterone nor by the inhibitors. Northern blot data are from Ref. 51.

cAMP production at the level of the adenylyl cyclase as well as upstream of it (63). Interestingly, the reverse, namely the control of aldosterone action by antidiuretic hormone, appears also to take place. Indeed, a recent study by Massaad et al. (28) indicates that cAMP increases via protein kinase A the number of MR which can be activated by aldosterone, possibly by relieving the effect of a MR repressor. This example of a reciprocal crosstalk demonstrates the intricate nature of the regulatory network that controls Na^+ transport and, in particular, ENaC function. It can be concluded that the functional impact on Na^+ transport of a single regulatory protein induced by aldosterone will depend on the cellular and physiological context. Thus, to understand the role of aldosterone-induced regulatory proteins such as K-Ras2 and sgk in the context of differentiated cells, it will be necessary to identify the downstream effectors that mediate their putative effect on Na^+ transport and to study the interactions of the different pathways finally acting directly or indirectly on the effectors of Na^+ transport.

EARLY REGULATION OF TRANSCRIPTION FACTORS BY ALDOSTERONE

Spindler and Verrey (51) have recently shown that aldosterone regulates the mRNA level of several transcription factors in A6 cells. The levels of c-*myc*, c-*jun*, c-*fos*, and GR mRNAs was decreased to 77–57% of the initial values within 2 h of treatment. After 16 h, c-*fos* and GR mRNA were further decreased while c-*jun* and c-*myc* returned toward control levels. Interestingly, this downregulation was shown for the three proto-oncogene mRNAs to be independent of ongoing transcription and, at least in part, independent of translation (Fig. 3). Thus this effect appears to be on mRNA stability, possibly by the activation of an AU-rich sequence-specific ribonuclease. This novel effect of aldosterone could be mediated by ligand-activated classic receptors (MR or GR), which would interact directly or indirectly with such a ribonuclease. Alternatively, the hsp complex that is released from the nuclear receptor during its ligand-induced activation could mediate this action, as previously shown for the activation of calcineurin in cortical collecting duct (57). Yet another possibility is that the effect on mRNA stability is mediated via a pathway independent of MR and GR, possibly by a membrane receptor (see above).

The decrease in c-*myc*, c-*jun*, and c-*fos* mRNAs can be seen in the context of the differentiating "anabolic" action of aldosterone. Indeed, Myc expression is known to inhibit terminal differentiation and to potentiate cell cycle progression. Jun and Fos also play essential roles in the control of cellular growth and differentiation as elements of the dimeric transcription factor AP-1. In contrast to the transcription factors mentioned above, Fos-related antigen 2 (Fra-2) was upregulated by aldosterone by a transcription-dependent mechanism. This can also be understood as promoting differentiation, since Fra-2 can associate with Jun instead of Fos and mediate suppression of transcription rather than transactivation (as would Fos).

ALDOSTERONE ACTION AT THE LEVEL OF THE SODIUM ION TRANSPORT EFFECTORS

Regulation of ENaC function. The nature of the early aldosterone action on ENaC, which is the major site of transepithelial Na^+ transport regulation, is not established. Noise analysis on A6 epithelia and patch-clamp experiments on rat cortical collecting duct principal cells indicate that the effect of aldosterone during its

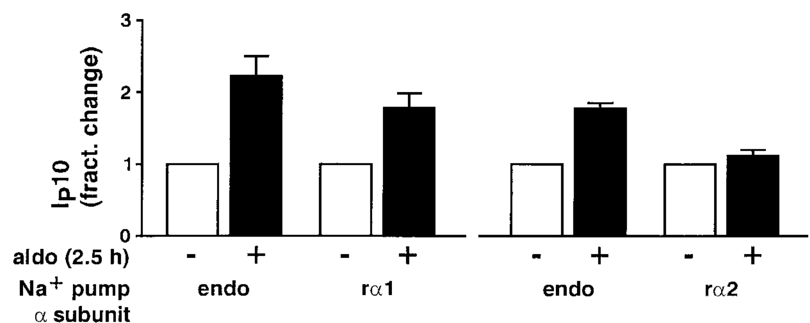

Fig. 4. Short-term aldosterone (2.5 h) increases the function of endogenous Na^+ pumps (endo) as well as Na^+ pumps containing transfected rat α1-subunits (rα1) in A6 epithelia. In contrast, Na^+ pumps containing α2-subunits (rα2) are not regulated. Na^+ pump function was measured as pump current (I_p10) in apically amphotericin B-permeabilized epithelia at a Na^+ concentration of 10 mM (modified from Ref. 39). Activated Na^+ pumps have a higher sensitivity to intracellular Na^+ than does the main Na^+ pump population (5).

early phase of action and in the long term consists of increasing the number of active channels (21, 37). However, since electrophysiological techniques do not "see" inactive channels, the question of whether new channels are brought to the surface or whether previously silent surface channels are activated cannot be answered by these techniques. As yet, this question has remained unanswered, as the tools available did not allow sufficiently specific quantitation of the channels expressed at the surface. In contrast to the studies mentioned above, a patch-clamp study on A6 epithelia, in which aldosterone was readded to epithelia from which it had been withdrawn a short time before, indicates that aldosterone increases the open probability of active channels (24). This apparent discrepancy could be explained by postulating that the acute aldosterone withdrawal performed in the latter case would induce a reversible state of low channel activity which is not observed otherwise. In any case, the question of how aldosterone acts on the channels is not yet answered. It could even be that aldosterone can act via different cascades, depending on the state of cellular differentiation (see above). The site of aldosterone action on the ENaC molecule and the type of posttranslational modification or of protein-protein interaction it produces are not yet elucidated.

The early regulation of Na^+ transport by aldosterone might depend in part on a methyl esterification reaction(s) (46). A recent study suggests that the enzyme which releases the end product inhibition of this methylation reaction, the *S*-adenosyl-L-homocysteine hydrolase, could be regulated at a posttranscriptional level by aldosterone (54). As yet, it is still controversial as to which proteins and/or lipids are the transmethylation substrates possibly of importance for the Na^+ transport response. The β-subunit of ENaC and the small G protein $p21^{ras}$ have been proposed to be such carboxymethylation substrates (43, 54). Aldosterone also appears to increase the GTPase activity of some membrane-associated proteins, including possibly that of $G\alpha_{i\text{-}3}$, but their functional role in the context of the Na^+ transport response is not clear (47).

ENaC is known to be regulated by many other pathways leading to different interactions and posttranslational modifications at the level of channel subunits such as binding of an ubiquitin ligase (Nedd4), ubiquitinylation, and direct phosphorylation (49, 52, 53). The channel activity might also be regulated by the action of an extracellular protease (known to activate the channel) (59). The readers are referred to excellent reviews on ENaC (16, 22) for further discussion of its structure and regulation.

Regulation of Na-K-ATPase function. The intracellular Na^+ concentration exerts a strong regulatory control on ENaC function (16, 22). This indicates that the function of the Na-K-ATPase, which controls the efflux of Na^+ from the cells, necessarily plays a major role in the control of Na^+ channel activity and thus transepithelial Na^+ transport (34). Aldosterone has been shown a long time ago to strongly and rapidly increase (within 1–3 h) Na-K-ATPase activity in kidney target epithelia of adrenalectomized animals (see bibliography of Ref. 62 for pertinent references). Based on the argument that aldosterone cannot increase that dramatically the number of pumps within this short time frame (pumps have a long half-life and are very numerous: $1–10 \times 10^6$ per cell), it has been postulated that it is the state of activity of the pumps which is regulated (62). In A6 epithelia, aldosterone has been shown to exert an action on the function of Na^+ pumps that might be of a similar nature. In this case, it appears that aldosterone activates only a small fraction of the pumps, which, however, have a higher affinity for Na^+ than the other pumps such that this effect leads to an approximately twofold increase in pump activity at physiological intracellular Na^+ concentrations (5). Experiments with A6 cell lines stably expressing different rat α-subunit isoforms have shown that this stimulatory action of aldosterone is restricted to pumps containing an α1-subunit (39) (Fig. 4). Thus it can be postulated that this regulatory action of aldosterone is mediated by a postranslational modification or a protein-protein interaction at the level of the α1-subunit of the Na-K-ATPase.

Regulation of K^+ channel function. As mentioned above, an increase in Na^+ reabsorption is necessarily coupled to an increase in apical and/or basolateral exit of K^+ due to the fact that the exchange of K^+ for Na^+ by the Na-K-ATPase must be compensated (19). It might be that aldosterone does not acutely regulate the function of apical K^+ channels during its early phase of action and that the early aldosterone-induced increase in K^+ secretion could be attributed to the increase in driving force due to the apical Na^+ conductance. In amphibian epithelia, in which there is no major apical secretion of K^+, aldosterone activates basolateral K^+ channels which have been shown in A6 cells to correspond to volume-activatable and quinidine-sensitive channels (9, 23). The nature of this regulation and whether it also takes place in mammalian target epithelia is not known.

OUTLOOK

New molecular techniques will soon lead to the identification of all early aldosterone-regulated gene products of some target epithelia. The remaining challenge will be to understand how the accumulation of these gene products leads to the Na^+ transport regulation. The identification of the first early aldosterone-regulated regulatory proteins already suggests that this will require understanding the interplay of different pathways which act on the function of effector channels and pumps. The available data also suggest that the limiting factors for Na^+ transport stimulation by aldosterone might not be the same in every target epithelium, depending on the differentiation of the regulatory network and the physiological context.

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REFERENCES

1. **Arriza, J. L., R. B. Simerly, L. W. Swanson, and R. M. Evans.** The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. *Neuron* 1: 887–900, 1988.
2. **Asher, C., H. Wald, B. C. Rossier, and H. Garty.** Aldosterone-induced increase in the abundance of Na^+ channel subunits. *Am. J. Physiol.* 271 (*Cell Physiol.* 40): C605–C611, 1996.
3. **Attali, B., H. Latter, N. Rachamim, and H. Garty.** A corticosteroid-induced gene expressing an "Isk-like" K^+ channel activity in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* 92: 6092–6096, 1995.
4. **Beesley, A. H., D. Hornby, and S. J. White.** Regulation of distal nephron K^+ channels (ROMK) mRNA expression by aldosterone in rat kidney. *J. Physiol. (Lond.)* 509: 629–634, 1998.
5. **Beron, J., L. Mastroberardino, A. Spillmann, and F. Verrey.** Aldosterone modulates sodium kinetics of Na,K-ATPase containing an alpha 1 subunit in A6 kidney cell epithelia. *Mol. Biol. Cell* 6: 261–271, 1995.
6. **Beron, J., and F. Verrey.** Aldosterone induces early activation and late accumulation of Na-K-ATPase at surface of A6 cells. *Am. J. Physiol.* 266 (*Cell Physiol.* 35): C1278–C1290, 1994.
7. **Bonvalet, J. P.** Regulation of sodium transport by steroid hormones. *Kidney Int.* 53, *Suppl.* 65: S49–S56, 1998.
8. **Bossuyt, X., M. Muller, B. Hagenbuch, and P. J. Meier.** Polyspecific drug and steroid clearance by an organic anion transporter of mammalian liver. *J. Pharmacol. Exp. Ther.* 276: 891–896, 1996.
9. **Broillet, M. C., A. Berger, and J. D. Horisberger.** Early effects of aldosterone on the basolateral potassium conductance of A6 cells. *Pflügers Arch.* 424: 91–93, 1993.
10. **Chen, S., A. Bhargava, L. Mastroberardino, O. C. Meijer, J. Wang, P. Buse, G. L. Firestone, F. Verrey, and D. Pearce.** Epithelial sodium channel regulated by aldosterone-induced protein sgk. *Proc. Natl. Acad. Sci. USA* 96: 2514–2519, 1999.
11. **DeRisi, J., L. Penland, P. O. Brown, M. L. Bittner, P. S. Meltzer, M. Ray, Y. Chen, Y. A. Su, and J. M. Trent.** Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat. Genet.* 14: 457–60, 1996.
12. **Doolan, C. M., and B. J. Harvey.** Modulation of cytosolic protein kinase C and calcium ion activity by steroid hormones in rat distal colon. *J. Biol. Chem.* 271: 8763–8767, 1996.
13. **Escoubet, B., C. Coureau, J. P. Bonvalet, and N. Farman.** Noncoordinate regulation of epithelial Na channel and Na pump subunit mRNAs in kidney and colon by aldosterone. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C1482–C1491, 1997.
14. **Fagart, J., J. M. Wurtz, A. Souque, C. Hellal-Levy, D. Moras, and M. E. Rafestin-Oblin.** Antagonism in the human mineralocorticoid receptor. *EMBO J.* 17: 3317–3325, 1998.
15. **Funder, J. W.** Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. *Annu. Rev. Med.* 48: 231–240, 1997.
16. **Garty, H., and L. G. Palmer.** Epithelial sodium channels: function, structure, and regulation. *Physiol. Rev.* 77: 359–396, 1997.
17. **Geering, K., M. Claire, H. P. Gaeggeler, and B. C. Rossier.** Receptor occupancy vs. induction of Na^+-K^+-ATPase and Na^+ transport by aldosterone. *Am. J. Physiol.* 248 (*Cell Physiol.* 17): C102–C108, 1985.
18. **Gekle, M., N. Golenhofen, H. Oberleithner, and S. Silbernagl.** Rapid activation of Na^+/H^+ exchange by aldosterone in renal epithelial cells requires Ca^{2+} and stimulation of a plasma membrane proton conductance. *Proc. Natl. Acad. Sci. USA* 93: 10500–10504, 1996.
19. **Giebisch, G.** Renal potassium transport: mechanisms and regulation. *Am. J. Physiol.* 274 (*Renal Physiol.* 43): F817–F833, 1998.
20. **Grunder, S., and B. R. Rossier.** A reappraisal of aldosterone effects on the kidney: new insights provide by epithelial sodium channel cloning. *Curr. Opin. Nephrol. Hypertens.* 6: 35–39, 1997.
21. **Helman, S. I., X. H. Liu, K. Baldwin, B. L. Blazer-Yost, and W. J. Els.** Time-dependent stimulation by aldosterone of blocker-sensitive ENaCs in A6 epithelia. *Am. J. Physiol.* 274 (*Cell Physiol.* 43): C947–C957, 1998.
22. **Horisberger, J. D.** Amiloride-sensitive Na channels. *Curr. Opin. Cell Biol.* 10: 443–449, 1998.
23. **Horisberger, J. D.** Early effects of aldosterone on apical and basolateral membrane conductances of TBM cells. *Am. J. Physiol.* 263 (*Cell Physiol.* 32): C384–C388, 1992.
24. **Kemendy, A. E., T. R. Kleyman, and D. C. Eaton.** Aldosterone alters the open probability of amiloride-blockable sodium channels in A6 epithelia. *Am. J. Physiol.* 263 (*Cell Physiol.* 32): C825–C837, 1992.
25. **Laplace, J. R., R. F. Husted, and J. B. Stokes.** Cellular responses to steroids in the enhancement of Na^+ transport by rat collecting duct cells in culture: differences between glucocorticoid and mineralocorticoid hormones. *J. Clin. Invest.* 90: 1370–1378, 1992.
26. **Liu, W., J. Wang, G. Y. Yu, and D. Pearce.** Steroid receptor transcriptional synergy is potentiated by disruption of the DNA binding domain dimer interface. *Mol. Endocrinol.* 10: 1399–1406, 1996.
27. **Liu, W. H., J. Wang, N. K. Sauter, and D. Pearce.** Steroid receptor heterodimerization demonstrated in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* 92: 12480–12484, 1995.
28. **Massaad, C., N. Houard, M. Lombes, and R. Barouki.** Modulation of human mineralocorticoid receptor function by protein kinase A. *Mol. Endocrinol.* 13: 57–65, 1999.
29. **Mastroberardino, L., B. Spindler, I. Forster, J. Loffing, R. Assandri, A. May, and F. Verrey.** Ras pathway activates epithelial Na^+ channel and decreases its surface expression in *Xenopus* oocytes. *Mol. Biol. Cell* 9: 3417–3427, 1998.
30. **Mastroberardino, L., B. Spindler, R. Pfeiffer, P. J. Skelly, J. Loffing, C. B. Shoemaker, and F. Verrey.** Amino acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature* 395: 288–291, 1998.
31. **May, A., A. Puoti, H. P. Gaeggeler, J. D. Horisberger, and B. C. Rossier.** Early effect of aldosterone on the rate of synthesis of the epithelial sodium channel alpha subunit in A6 renal cells. *J. Am. Soc. Nephrol.* 8: 1813–1822, 1997.
32. **McEwan, I. J., A. P. H. Wright, and J. A. Gustafsson.** Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. *Bioessays* 19: 153–160, 1997.
33. **Meyer, T., D. B. Starr, and J. Carlstedt-Duke.** The rat glucocorticoid receptor mutant k461a differentiates between two different mechanisms of transrepression. *J. Biol. Chem.* 272: 21090–21095, 1997.
34. **Muto, S., Y. Asano, D. Seldin, and G. Giebisch.** Basolateral Na^+ pump modulates apical Na^+ and K^+ conductances in rabbit cortical collecting ducts. *Am. J. Physiol.* 276 (*Renal Physiol.* 45): F143–F158, 1999.
35. **Naray-Fejes-Toth, A., I. K. Colombowala, and G. Fejes-Toth.** The role of 11β-hydroxysteroid dehydrogenase in steroid hormone specificity. *J. Steroid Biochem. Mol. Biol.* 65: 311–316, 1998.
36. **Paccolat, M. P., K. Geering, H. P. Gaeggeler, and B. C. Rossier.** Aldosterone regulation of Na^+ transport and Na^+-K^+-ATPase in A6 cells: role of growth conditions. *Am. J. Physiol.* 252 (*Cell Physiol.* 21): C468–C476, 1987.
37. **Pacha, J., G. Frindt, L. Antonian, R. B. Silver, and L. G. Palmer.** Regulation of Na channels of the rat cortical collecting tubule by aldosterone. *J. Gen. Physiol.* 102: 25–42, 1993.
38. **Pearce, D., and K. R. Yamamoto.** Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science* 259: 1161–1165, 1993.
39. **Pfeiffer, R., and F. Verrey.** Regulation of Na^+ pump function by aldosterone is α-subunit isoform-specific. *J. Physiol.* 516: 647–655, 1999.
40. **Rachamim, N., H. Latter, N. Malinin, C. Asher, H. Wald, and H. Garty.** Dexamethasone enhances expression of mitochondrial oxidative phosphorylation genes in rat distal colon. *Am. J. Physiol.* 269 (*Cell Physiol.* 38): C1305–C1310, 1995.
41. **Reichardt, H. M., K. H. Kaestner, J. Tuckermann, O. Kretz, O. Wessely, R. Bock, P. Gass, W. Schmid, P. Herrlich, P. Angel, and G. Schütz.** DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* 93: 531–541, 1998.

42. **Renard, S., N. Voilley, F. Bassilana, M. Lazdunski, and P. Barbry.** Localization and regulation by steroids of the alpha, beta and gamma subunits of the amiloride sensitive Na^+ channel in colon, lung and kidney. *Pflügers Arch.* 430: 299–307, 1995.
43. **Rokaw, M., J. Wang, R. Edinger, O. Weisz, D. Hui, P. Middleton, V. Shlyonsky, B. Berdiev, I. Ismailov, D. Eaton, D. Benos, and J. Johnson.** Carboxylmethylation of the beta subunit of xENaC regulates channel activity. *J. Biol. Chem.* 273: 28746–28751, 1998.
44. **Rupprecht, R., J. L. Arriza, D. Spengler, J. M. H. M. Reul, R. M. Evans, F. Holsboer, and K. Damm.** Transactivation and synergistic properties of the mineralocorticoid receptor: relationship to the glucocorticoid receptor. *Mol. Endocrinol.* 7: 597–603, 1993.
45. **Rupprecht, R., J. M. H. M. Reul, B. Vansteensel, D. Spengler, M. Soder, B. Berning, F. Holsboer, and K. Damm.** Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands. *Eur. J. Pharmacol.* 247: 145–154, 1993.
46. **Sariban-Sohraby, S., M. Burg, W. P. Wiesmann, P. K. Chiang, and J. P. Johnson.** Methylation increases sodium transport into A6 apical membrane vesicles: possible mode of action of aldosterone action. *Science* 225: 745–746, 1984.
47. **Sariban-Sohraby, S., M. Svoboda, and F. Mies.** Guanine nucleotide binding proteins in cultured renal epithelia: studies with pertussis toxin and aldosterone. *Am. J. Physiol.* 276 (*Renal Physiol.* 45): F10–F17, 1999.
48. **Schmidt, T. J., R. F. Husted, and J. B. Stokes.** Steroid hormone stimulation of Na^+ transport in A6 cells is mediated via glucocorticoid receptors. *Am. J. Physiol.* 264 (*Cell Physiol.* 33): C875–C884, 1993.
49. **Shimkets, R. A., R. Lifton, and C. M. Canessa.** In vivo phosphorylation of the epithelial sodium channel. *Proc. Natl. Acad. Sci. USA* 95: 3301–3305, 1998.
50. **Spindler, B., L. Mastroberardino, M. Custer, and F. Verrey.** Characterization of early aldosterone-induced RNAs identified in A6 kidney epithelia. *Pflügers Arch.* 434: 323–331, 1997.
51. **Spindler, B., and F. Verrey.** Aldosterone action: induction of $p21^{ras}$ and *fra*-2 and transcription-independent decrease in *myc*, *jun*, and *fos*. *Am. J. Physiol.* 276 (*Cell Physiol.* 45): C1154–C1161, 1999.
52. **Staub, O., S. Dho, P. C. Henry, J. Correa, T. Ishikawa, J. McGlade, and D. Rotin.** WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na^+ channel deleted in Liddle's syndrome. *EMBO J.* 15: 2371–2380, 1996.
53. **Staub, O., I. Gautschi, T. Ishikawa, K. Breitschopf, A. Ciechanover, L. Schild, and D. Rotin.** Regulation of stability and function of the epithelial Na^+ channel (ENaC) by ubiquitination. *EMBO J.* 16: 6325–6336, 1997.
54. **Stockand, J., N. Al-Baldawi, O. Al-Khalili, R. Worrell, and D. Eaton.** *S*-adenosyl-L-homocysteine hydrolase regulates aldosterone-induced Na^+ transport. *J. Biol. Chem.* 274: 3842–3850, 1999.
55. **Stoecklin, E., M. Wissler, R. Moriggl, and B. Groner.** Specific DNA binding of STAT5, but not of glucocorticoid receptor, is required for their functional cooperation in the regulation of gene transcription. *Mol. Cell. Biol.* 17: 6708–6716, 1997.
56. **Trapp, T., R. Rupprecht, M. Castren, J. M. H. M. Reul, and F. Holsboer.** Heterodimerization between mineralocorticoid and glucocorticoid receptor: a new principle of glucocorticoid action in the CNS. *Neuron* 13: 1457–1462, 1994.
57. **Tumlin, J. A., J. P. Lea, C. E. Swanson, C. L. Smith, S. S. Edge, and J. S. Someren.** Aldosterone and dexamethasone stimulate calcineurin activity through a transcription-independent mechanism involving steroid receptor-associated heat shock proteins. *J. Clin. Invest.* 99: 1217–1223, 1997.
58. **Ueda, K., N. Okamura, M. Hirai, Y. Tanigawara, T. Saeki, N. Kioka, T. Komano, and R. Hori.** Human p-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.* 267: 24248–24252, 1992.
59. **Vallet, V., A. Chraibi, H. P. Gaeggeler, J. D. Horisberger, and B. C. Rossier.** An epithelial serine protease activates the amiloride-sensitive sodium channel. *Nature* 389: 607–610, 1997.
60. **Verrey, F.** Early aldosterone effects. *Exp. Nephrol.* 6: 294–301, 1998.
61. **Verrey, F.** Transcriptional control of sodium transport in tight epithelia by adrenal steroids. *J. Membr. Biol.* 144: 93–110, 1995.
62. **Verrey, F., J. Beron, and B. Spindler.** Corticosteroid regulation of renal Na,K-ATPase. *Miner. Electrolyte Metab.* 22: 279–292, 1996.
63. **Verrey, F., M. Digicaylioglu, and U. Bolliger.** Polarized membrane movements in A6 kidney cells are regulated by aldosterone and vasopressin/vasotocin. *J. Membr. Biol.* 133: 213–226, 1993.
64. **Verrey, F., J. P. Kraehenbuhl, and B. C. Rossier.** Aldosterone induces a rapid increase in the rate of Na,K-ATPase gene transcription in cultured kidney cells. *Mol. Endocrinol.* 3: 1369–1376, 1989.
65. **Verrey, F. V., E. Hummler, L. Schild, and B. C. Rossier.** Control of Na^+ transport by aldosterone. In: *The Kidney, Physiology and Pathophysiology* (3rd ed.), edited by D. W. Seldin and G. Giebiesch. New York: Raven. In press.
66. **Wehling, M.** Specific, nongenomic actions of steroid hormones. *Annu. Rev. Physiol.* 59: 365–393, 1997.
67. **Wehling, M., C. Spes, N. Win, C. Janson, B. Schmidt, K. Theisen, and M. Christ.** Rapid cardiovascular action of aldosterone in man. *J. Clin. Endocrinol. Metab.* 83: 3517–3522, 1998.
68. **Zhang, L., W. Zhou, V. E. Velculescu, S. E. Kern, R. H. Hruban, S. R. Hamilton, B. Vogelstein, and K. W. Kinzler.** Gene expression profiles in normal and cancer cells. *Science* 276: 1268–1272, 1997.