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Topical Review

Transcriptional Control of Sodium Transport in Tight Epithelia by Adrenal Steroids

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Introduction

Aldosterone Action: Levels of Consideration

Extracellular volume and osmolarity are maintained within a narrow range by the interplay of a number of intricate hormonal and autoregulatory systems [52]. The kidney and to some extent the urinary bladder, the intestine, sweat glands, as well as the skin in amphibia, play an important role for this homeostasis as effectors of fluid and ion output. It has long been recognized that aldosterone has a potent sodium-retaining activity which is mainly due to its action at the level of the electrically "tight" epithelia of these organs (*see* for review and older references: [9, 25, 33, 42, 45, 61, 63, 76, 98, 99]). At the level of these epithelia it stimulates Na reabsorption which takes place across the cells by a two-step mechanism involving an apical amiloride-sensitive Na channel and the basolateral Na pump (Na,K-ATPase). The Na,K-ATPase appears to be composed of $\alpha 1$ and $\beta 1$ subunits [127, 136] and the epithelial Na channel is of the high selectivity/low conductance (4–5 pS) type for which three homologous subunits have recently been cloned from a rat distal colon library [23, 83, 97].

Besides its action on sodium retention, aldosterone also exerts a major effect on potassium secretion and pH control. This review will focus on the regulation of Na reabsorption by aldosterone and other adrenal steroids.

In a reductionist approach, the action of aldosterone has progressively been investigated on hierarchically lower level systems such as the whole kidney, single nephron segments, isolated tubules and flat native or cultured epithelia, single cells, individual membranes and finally single transport molecules. The use of techniques originating from biochemistry, cell biology and molecular biology on relatively homogenous sources of target cells such as tissue cultures or isolated tubules has provided insight into the structure and regulation of proteins known to be involved in hormonally regulated Na reabsorption.

As a consequence of these multiple approaches, we are now confronted with a wealth of information obtained at different levels of consideration and using experimental systems originating from different species and target tissues as well as different experimental conditions. The integration of this information is further complicated by the fact that observations made on a given scale cannot always be transferred to another scale without the addition of other information [112]. In this context the cellular level plays a pivotal role as a meeting point between smaller scale and larger scale observations as well as molecular and physiological perspectives.

The "Historical" Model for the Cellular Action of Aldosterone

Edelman and colleagues introduced a model for the cellular action of aldosterone on Na transport across tight epithelia 30 year ago [35]. This model proposed that the action of aldosterone is mediated by the transcriptional regulation of a set of genes which code for proteins (aldosterone-induced proteins, AIPs) which are responsible for enhanced transcellular ion transport. Though this model has been refined to some extent [45, 98, 99, 124], it still represents the conceptual framework in which new experimental results are tentatively organized.

Key words: Aldosterone—Mineralocorticoid hormone—Glucocorticoid hormone—Hormonal regulation—Na^+ channel—Na^+,K^+-ATPase

Although it has generally been accepted that the physiological response of tight epithelia to adrenal steroids is mediated by the transcriptional effect of the hormones, it is still an open question as to how this action results in the known changes at the level of the ion transport proteins. Indeed, hormonally regulated proteins which mediate the early response have not been identified to date (*see below*).

The aim of this review is to revisit and expand the cellular model for aldosterone action in order to better integrate and organize recent and future experimental data. These new data, to a large extent, come from two complementary approaches which tend to fill the gap of understanding that lies between the transcriptional events and the functional response. On the one hand, refinements at the level of the classical physiological approach, such as the use of better defined experimental models or new techniques (e.g., patch clamp) have permitted a more detailed analysis of the functional response at the level of effectors. In such studies numerous known intracellular mediators have been tested for their possible involvement in the mediation of the adrenal steroid action. On the other hand, much has been learned about the regulatory action of steroid hormones at the transcriptional level and the search for genes regulated by adrenal steroid hormones has become much easier. It can therefore be expected that a large number of regulated mRNAs will be identified in the near future. However, these will be identified in various systems and after the administration of different hormones given at different concentrations and for different times. It will therefore become central to have the means to sort out and to identify those gene products which participate directly or indirectly in the physiological response.

Much of the data on Na transport regulation by adrenal steroids discussed in this review and considered for the extended model of adrenal steroid action have been obtained using amphibian systems and in particular the A6 cell line. This is not only due to the fact that the author works with A6 cells but it is because these experimental systems with their relative simplicity have led over the past decades to many advances in the understanding of the cellular and molecular mechanisms of adrenal steroid action on sodium reabsorption. Since many of the features first described in amphibian systems appear to be conserved in avian and mammalian tight epithelia, it is reasonable to expect that the model discussed here should be useful for the interpretation of data obtained in the avian and mammalian systems.

It has to be mentioned that aldosterone has been shown to have, in certain cells, a "nongenomic" effect which appears to be mediated by a membrane receptor and involves the stimulation of the Na/H exchanger [80, 130, 135]. However, although cellular pH plays an important role in Na transport regulation [7, 24, 54, 83], as yet no report has conclusively implicated such a "nongenomic" mechanism of adrenal steroids in the stimulation of Na transport across tight epithelia.

Steroid Receptors: Ligand Activated Transcription Factors with Modular Organization

Before discussing the physiological response of tight epithelia to adrenal steroids and addressing the question of which genes are regulated and play a role in that response, it appears appropriate to first consider those events by which a change in extracellular hormone concentration produces a target cell-specific change in gene transcription.

The receptors for adrenal steroid hormones belong to a family of ligand-activated nuclear hormone receptors and hence the understanding of their general features stems from studies performed on the various family members [37]. The cascade of events which is produced by the ligand can schematically be described as follows (*see* Fig. 1). Free adrenal steroid hormone penetrates the target cell and binds in the cytosol to its receptors. The binding facilitates the dissociation of the receptor from a protein complex containing hsp90, thereby exposing nuclear localization sequences and the DNA binding domain such that homodimerization and translocation to the nucleus can take place [2, 12, 58, 92, 108, 120]. There, the activated receptors can bind to specific DNA (recognition) sequences (hormone response elements, HRE's) within the control elements of regulatable genes and affect the rate of transcription of these target genes.

Every type of nuclear hormone receptor modulates the transcription of a specific but generally overlapping set of genes in a given cell (hormonal domain) [60, 117]. The specificity is generated at the level of various steps along the cascade of events mentioned above and by the cell lineage and differentiation-specific accessibility of regulatory sequences which is controlled by the state of the chromatin, and by the presence of general and cell-specific transcription factors implicated in the modulation of the transcriptional activity by the hormone-receptor complex. The question of specificity is of particular interest for the adrenal steroid action on ion transport. Indeed, despite clear differences of the response to mineralocorticoid and glucocorticoid hormones at the systemic and organ levels, the precise role of the two receptors (type I or mineralocorticoid (MR) and type II or glucocorticoid receptor (GR)), the extent of their functional overlap and the role of potential specificity-conferring mechanisms are not yet fully understood at the cellular level.

Structurally, all members of the nuclear receptor family, which are ligand-activatable transcription factors, share a common basic modular organization. Several different functions, many of which will be mentioned below, have been attributed to specific domains

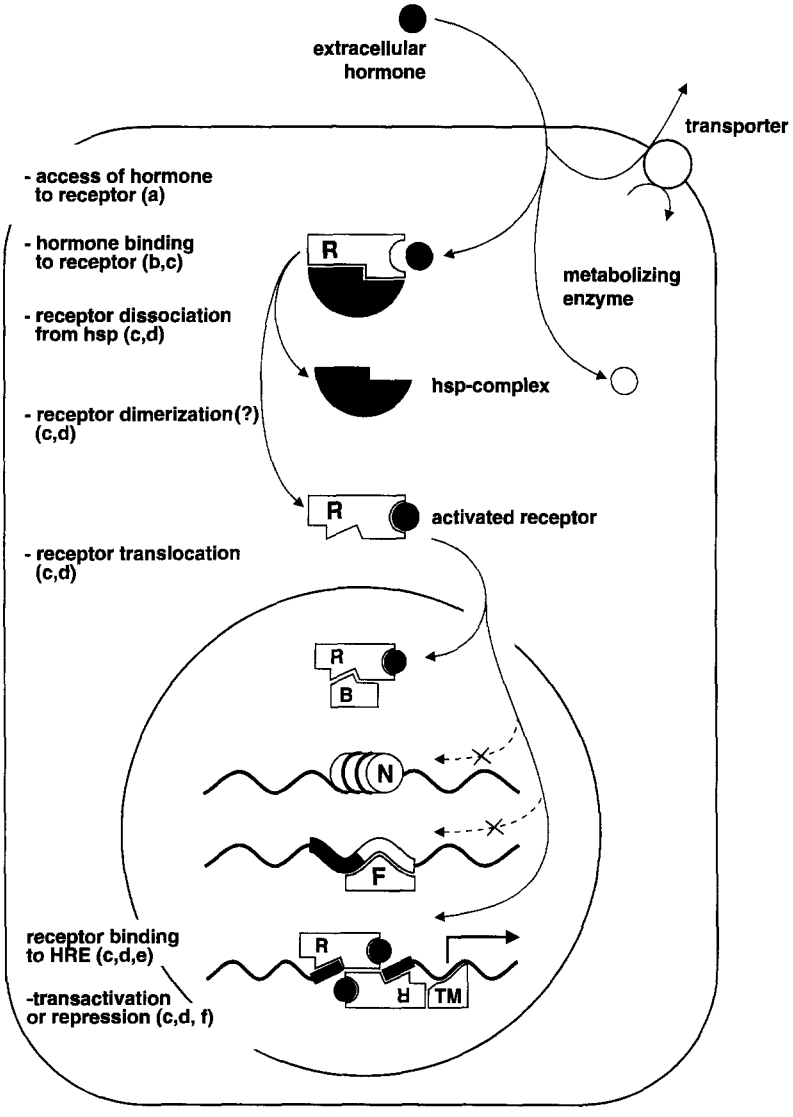

Fig. 1. Mechanism of adrenal steroid action: Sequence of events leading to gene regulation and possible (specificity-)control mechanisms. The letter (*a–f*) indicate possible control mechanisms which might confer a receptor and/or hormone specificity to the transcriptional regulation of a gene by steroid hormones. (*a*) Prereceptor barriers for hormones: hormone metabolism (e.g., 11β-OHSDH); hormone transport (e.g., MDR). (*b*) Receptor expression: transcriptional and post-transcriptional control. (*c*) Ligand-independent functional state of receptor: covalent modifications (e.g., phosphorylation); noncovalent interactions. (*d*) Ligand-dependent functional state of receptor: differential effect of different agonists, antagonists, partial (ant)agonists. (*e*) Access to hormone regulatory elements (HRE's) of potentially regulated genes: receptor sequestration, chromatin structure, steric hindrance by other transcription factor, competition with other factor. (*f*) Effect at regulatable genes: positive or negative interactions with other factors and/or transcription machinery and/or homologous or heterologous receptors. *R* stands for receptor, *B* for receptor binding protein, *N* for nucleosome, *F* for transcription factor and *TM* for basal transcription machinery.

and subdomains [37, 120]. The three major domains of these receptors and their associated functions shall briefly be mentioned here:

(i) The C-terminal domain is referred to as the hormone-binding domain but also plays a major role for the binding of heat shock proteins, receptor dimerization, nuclear localization, interaction with other factors, modulation of DNA binding and activation of gene transcription (transactivation) [67, 120, 140]. The general organization of this domain appears to be conserved between receptors and its level of identity between the two adrenal steroid receptors is 57% [37].

(ii) A central DNA binding domain linked to the hormone binding domain by the "hinge region" is composed of two Cys-Cys zinc fingers and is highly conserved among all receptors (94% identity between gluco- and mineralocorticoid receptors [37]). The three-dimensional structure of this domain bound to specific DNA sequences has been determined from crystals and in solution, and the specific amino acid-DNA interactions have been mapped [11, 72, 107]. It is important to remember that the nuclear receptor family can be divided into only two classes of receptors which recognize two different DNA motifs. The lack of specificity of these hormone response elements (HRE's) indicates that other features must underly the specificity of hormone action. Both adrenal corticoid receptors belong to the same class of receptors which, like androgen and progesterone receptors (AR and PR), can bind to and transactivate from imperfect palindromic or half-palindromic structures with the consensus sequence (GGTACAnnn)TGTYCY. The receptors for estrogen (ER), thyroid hormones, vitamin D3 and retinoic acids belong to the other class and recognize a consensus sequence GGTCAnnnTGACC [120]. The DNA binding domain also appears to play an important role for the homodimerization of the receptors

and might also interact with other transcription factors and provide a (weak) transactivation function.

(iii) The N-terminal domain is very different between receptors such that no significant sequence identity has been identified in this domain between the related gluco- and mineralocorticoid receptors (less than 15% identity [37]). A promoter- and cell-specific transactivating function has been localized to this domain [16, 27, 120]. This variability might be related to the fact that this domain plays a crucial role in the receptor-specific interplay with homologous receptors or other transcription factors and components of the basal transcription machinery.

Hormones, Receptors and Specificity-Conferring Mechanism

That mineralocorticoid and glucocorticoid hormones have different physiological effects at the systemic level, in particular on the ion balance, is well known ([25, 42, 76] and references therein). However, to understand how this difference is generated has become a challenge for the investigators because of an apparent lack of specificity in the transduction pathway downstream of the hormones. Indeed, it has become clear that the two hormones can act via the same (two) receptors which, furthermore, can both bind to the same hormone recognition elements (HRE) of target genes and exert similar transactivation functions [3, 43, 100, 101].

Specifically, the major glucocorticoid hormone cortisol (or corticosterone in rats and mice) binds with a similar high affinity to the type I (mineralocorticoid) receptor (MR) as the mineralocorticoid hormone aldosterone [3]. Aldosterone also binds to the type II or glucocorticoid receptor (GR) with an affinity similar to that of cortisol [101]. However, in physiological situations the concentration of circulating cortisol is much higher than that of aldosterone such that it should (despite its higher binding to serum proteins) nearly saturate the binding capacity of the MR while aldosterone would occupy an insignificant proportion of both receptors.

It therefore appears that hormone and receptor specificity are generated at multiple levels and in a cell-specific manner. The aim in this section is to summarize the potential and established specificity-conferring mechanisms, following the sequence of events schematically represented in Fig. 1.

Prereceptor Barriers for Hormones

Much emphasis has recently been given to the barrier formed in aldosterone target cells by an 11β-hydroxysteroid dehydrogenase (11β-OHSDH) which can prevent the occupation of adrenal steroid receptors (MR and GR) by endogenous cortisol or corticosterone. Indeed, a NAD-preferring "isoform" of the enzyme, which preferentially catalyzes the "forward" reaction by which the hormones are converted to receptor-inactive metabolites, is expressed in many classical aldosterone target cells [19, 42, 66, 79, 132]. This barrier is selective because the C-11 hydroxyl group of aldosterone forms a hemiketal with the C-18 aldehyde and thus is resistant to dehydrogenation. That this "protection" mechanism plays an important physiological role in vivo was demonstrated by the correlation of the inhibitory action of glycyrrhetinic acid on the metabolism of cortisol by the 11β-OHSDH and its salt-retaining and hypertensive actions [113].

The effect of the 11β-OHSDH has attracted much attention because it gives a unitary explanation to the capability of aldosterone to act via the MR which otherwise would be occupied (some say illegitimately) and activated by cortisol. However, this protection mechanism probably does not suffice to explain how the MR is protected from other endogenous (partial) agonists or inhibitors such as deoxycorticosterone and progesterone [42, 101]. Furthermore, it remains to be demonstrated how absolute this barrier is in the different aldosterone target tissues. For instance, it will be interesting to understand its role in the distal colon, where glucocorticoids and aldosterone play a physiologically reciprocal role in the differential regulation of two Na absorptive mechanisms [10]. It will also be interesting to understand to what extent the regulation of the 11β-OHSDH might introduce a dynamic control of the access of cortisol to the MR. That its expression is regulated and is induced by its own substrate hormone has been demonstrated in amphibian urinary bladder cells [44].

The 11β-OHSDH is not the unique steroid metabolizing enzyme potentially present in target cells. It can therefore be expected that local metabolism might play other important roles in controlling steroid hormone action. For instance, the conversion of hormones into active metabolites has been implicated as well. Indeed, it has been shown in amphibian bladders and A6 cells that endogenously produced metabolites of corticosterone have an activity on Na transport [34, 102].

Certain steroids appear to be substrates for the P-glycoprotein which is the product of the multidrug resistance (MDR) gene. It has, for example, been demonstrated that cortisol and aldosterone (to a lesser extent) are transported by the P-glycoprotein while progesterone is not [123]. Functionally, P-glycoprotein expression has been associated with resistance to the apoptotic action of glucocorticoids in certain cells [17]. Whether such transport systems play a physiological role in the modulation of adrenal steroid action in target cells is not known. It is however interesting to mention that A6 cells express a P-glycoprotein type transport system, presumably in the apical membrane, since typical substrates are

effectively transported vectorially from the basolateral to the apical medium (A. Spillmann and F. Verrey, *unpublished results*).

In summary, though it is now well known that local hormone metabolism plays a crucial role in determining the accessibility of receptors, several other mechanisms might exist which control the hormone action by modulating the accessibility of the receptors for the hormone.

Expression Level and Functional State of Receptors

It appears that the action of aldosterone via nuclear receptors is mediated by the MR since the concentration of this hormone and its affinity for the GR are, in physiological conditions, not compatible with a significant binding to this second receptor. Therefore, potential target genes will only be regulatable by the aldosterone-MR complex in a restricted cell population since, unlike the GR, the MR is not ubiquitously expressed. This feature confers cell-specificity to aldosterone action [3, 42, 71, 99].

An example of a Na reabsorbing epithelium in which the level of MR expression relative to the GR might control the expression of specific genes in otherwise similar cells is that of the colon. It has been proposed that the differential expression of Na transporting systems along this organ could be due to the increase in MR expression from its proximal to distal part [10]. How, in turn, the expression of the receptors might be controlled is not known. In general, it is expected that the cell-specific developmental control of the receptor expression is based on the same principles as that of other genes [38].

A degree of cell specificity and/or a control of the hormone and receptor specificity could be introduced by a differential tuning of the functional state of receptors mediated by covalent modifications or noncovalent interactions [57, 69, 91, 120, 122]. An example is given by the results of Power et al. [91] which studied the ligand-independent activation of various steroid nuclear receptors expressed in CV_1 cells. Interestingly, upon exposure to dopamine some receptors were activated, some not. Specifically, while dopamine treatment did not affect the GR action, the MR was activated, though only marginally. In this case a difference in receptor activation is probably created by a differential phosphorylation of the steroid receptors by the same signal transduction cascade. Clearly, besides the fact that receptor phosphorylation can provide a means of crosstalk with other signaling pathways and cell cycle dependent regulation, it could also interfere with the hormone and receptor specificity.

Ligand-Specific Receptor Function

In addition to its physiological importance, the ligand-specificity of the various functional states of these receptors has great pharmacological relevance. Indeed, ligand binding *per se* does not always switch the receptor from a unique inactive state to a unique active one. On the contrary, it is well known that some ligands have partial agonistic and/or antagonistic activities. It is believed that this type of variability depends on differential conformational changes induced in the receptor by the binding of various ligands. It has also been postulated that the ligand *per se* might be directly involved in a receptor function; for instance, it could be part of the binding interface for receptor dimerization [75, 120]. Interestingly, different ligands can produce differential effects at the level of various steps in the cascade depicted in Fig. 1, hence there are numerous possibilities of general, cell- or gene-restricted mechanisms for hormone specificity-conferring mechanisms.

A first level of ligand-specific action is the differential stability of cytoplasmic receptor complexes. Indeed, the dissociation rate of the ligand from the receptor, in the presence or in the absence of the hsp complex, depends on the nature of the ligand. A short half-life of the ligand-receptor complex might favor an interruption in the sequence of events which lead to gene regulation. It has also been shown that the nature of the ligand affects the dissociation rate of the receptor from the hsp complex. Interestingly, in the case of the MR this dissociation was faster in the presence of an antagonist than in the presence of aldosterone [93].

Another function of the receptors which has, in some instances, been shown to depend on the nature of the ligand bound is the ability of activated receptors to translocate to the nucleus [28, 74]. In this respect it has to be mentioned that generally two classes of nuclear receptors are discriminated based on their subcellular localization in the unliganded state: the "nuclear" (ER, PR, AR) and the "cytoplasmic" receptors (GR, MR). Recent experiments have, to some extent, challenged this view and it now appears that this difference is not absolute. Both "nuclear" and "cytoplasmic" receptors would shuttle back and forth, from and to the cytoplasm, the latter at least in their activated state [28, 51, 92]. The difference between the two classes of receptors could be quantitative and due to receptor-specific differences in exposure of the nuclear localization signal(s) in the hsp-bound nonactivated state. In the case of the "cytoplasmic" receptors the nuclear localization signal(s) would be masked by the associated hsp90 in the absence of bound ligand, whereas that of the "nuclear" receptors would be exposed, such that the entire complex could be concentrated in the nucleus [64]. An interesting example of the differential effect of ligands on receptor translocation is that of the ER which has been shown to localize to the cytoplasm in the presence of an antihormone (ICI 182780) while otherwise it is mostly nuclear, even in the absence of ligand [28].

The dimerization and DNA binding functions of nu-

clear receptors have also been shown to depend on the nature of the ligand in some instances [2, 75].

That the ligand-induced conformational change plays a major role for the transactivation activity of a receptor *per se* can be inferred from experiments of the group of O'Malley with the PR and the ER [2, 12]. However, it has to be remembered that ligand-dependent variability in gene expression does not necessarily result from differences in transactivation function *per se*. Indeed, ligand-specific differences in transcriptional regulation by the same receptor can also result from ligand-specific effects at any of the levels upstream of transactivation mentioned above. Furthermore, the accumulation of the products (mRNA or protein) can also be differentially affected.

Potential ligand-specific differences in transactivation by the MR were investigated by transfection experiments [3, 101]. The action of the MR on the transcription of a reporter gene with MMTV regulatory sequences was tested in the presence of various ligands. Interestingly, though the maximal transactivation effect by the hMR was similar in the presence of aldosterone or deoxycorticosterone, this second hormone had a lower potency for transactivation than aldosterone, despite a nearly tenfold higher affinity for the MR. Clearly in this case a "functional preference" for aldosterone generates a hormone specificity despite the "use" of a common receptor. This is just a single example with a particular gene in a particular cell type. It can be expected, however, that such differential effects of ligands can be found at the level of many genes and in many cell types and therefore might play a crucial role in determining the specificity of the hormone action. It is important to note that in the case presented above the difference observed between the two ligands was not at the level of the maximal effect but at that of the $K_{0.5}$ such that it would have been missed in experiments in which only maximal effects are measured. It can be concluded that this level of specificity is important in vivo but that its study is complicated by the fact that it might be gene- and cell-specific and because it is visible at physiological but not at pharmacological hormone concentrations.

Accessibility of HRE's

To be regulatable by a hormone-receptor complex, a gene has not only to have (an) adequate HRE('s) but this also has to be accessible. Here, the chromatin organization plays a crucial role in determining the network of genes available for regulatable expression [139]. It shall only be mentioned that this level of organization is cell-lineage and differentiation-specific and that the action of an activated receptor might, in some instances, depend on the presence of "chromatin modifying factors" which act on the chromatin structure (as opposed to transcription factors which interact with the basal transcription machinery) [77, 121, 142]. Steroid receptors themselves have been shown to interfere at the level of the chromatin organization in some cases. An example is that of the mouse mammary tumor virus (MMTV) promoter where the activated GR modifies the interaction of the DNA with the nucleosome by its binding to the HRE such that the binding site of another nuclear factor becomes accessible and this factor can then activate transcription [119, 120, 139]. Hormone specificity might also be generated by the accessibility to regulatory sequences since, in some instances, response elements might be exposed which preferentially respond to one or the other receptor (e.g., MR vs. GR).

It might also be that, in addition to the potential interference of chromatin structure with receptor binding to "its" HRE, other proteins which bind close to or at the HRE sterically hinder the receptor binding [120]. Such a competitive situation for DNA binding clearly depends on the sequence context of the HRE and on the cell-specific level of expression of such binding proteins. Furthermore, this type of competition at the HRE might also be receptor-specific and thereby represent a specificity-conferring mechanism.

Binding of the activated receptors to proteins outside of the context of the HRE has also been proposed as a mechanism to prevent access of receptors to potentially regulated genes. Two examples are the calcium binding protein calreticulin and the transcription factor subunit NFkappaB p65 [21, 29, 94]. Since this binding could also be receptor-specific, such a "sequestration" of a receptor could render a potential target gene of two receptors *de facto* specific for one.

Transactivation and Repression

A first important observation is that the degree of transactivation by different receptors of the same family (e.g., GR, MR, PR and AR) which bind onto the same HRE of a target gene is not necessarily identical. For instance, the maximal transcription rate induced by the MR is clearly lower than that produced by the GR when tested in transfection assays on several different reporter genes containing glucocorticoid regulatory elements [3, 100, 101]. It appears that the main difference is due to the lower transactivation function of the amino-terminal domain of the type I receptor. This domain also prevents the synergistic effect on transcription (cooperativity) observed with promoters containing multiple palindromic GRE's, an effect which can be mediated by the DNA- and ligand-binding domains of either receptor [100]. Based on these observations it has been proposed that the mineralocorticoid receptor could extend the dynamic range of the glucocorticoid hormone action in cells which express both receptors. This receptor, be-

cause of its high affinity for endogenous glucocorticoids and its relatively low transactivating activity, would mediate the glucocorticoid action on glucocorticoid-responsive genes at low hormone concentrations [3]. Such a role has been proposed for the neurons of the hippocampus while in the hypothalamus the expression of specificity-conferring mechanisms would confer a more specific action to aldosterone comparable to the situation in distal nephron cells [30].

In view of the possible specificity-conferring mechanisms mentioned above, it is also possible that there is a set of as yet unidentified genes with complex regulatory elements at which the MR produces a higher transactivation or repression than the GR (receptor specificity: *see e.g.,* [39]). A level of hormone specificity could be conferred to these putative MR-specific elements if the transactivation was dependent on the ligand bound to the receptor.

It remains, however, that many potential MR target genes can be regulated by the MR activated by glucocorticoid hormone as well as by the GR. Furthermore, that the activation of the MR by the endogenous glucocorticoid hormone has a physiological relevance, at least for certain cells, is supported by the fact that in species in which the major glucocorticoid hormone is corticosterone (rat and mouse) this hormone has a higher affinity for the MR than cortisol, whereas in those species which have cortisol as their major glucocorticoid hormone, cortisol has a higher affinity for the MR ([101] and references therein).

A second important observation is that differences in transactivation by receptors of the same family (e.g., GR, MR, PR and AR) are gene-specific and depend on nonreceptor factors. An example is the specificity of the transactivation by androgens at the sex-limited protein gene. Indeed, although GR can bind to the HRE of this gene, transactivation is only produced by the AR. The specificity-conferring differential effect has been mapped to the N-terminal domain of the AR and was attributed to nonreceptor factors which specifically "cooperate" with the AR [1]. Another example is that of repression by the GR and not by the MR of AP-1 (with a given ratio of cJun and cFos) stimulated transcription of the proliferin gene. In this case the mechanism conferring receptor specificity was also mapped to the N-terminus of the receptor and was also due to the interaction (in this case negative) at the response element with a nonreceptor factor [88].

In summary, it appears that a receptor-specificity is conferred onto complex response elements with similar recognition sequences for nuclear steroid receptors by the presence of binding sites for other factors which differentially interact with the various hormone receptors. Such specificity-conferring mechanisms could be expressed in a cell-specific way, since they depend on the presence of other (cell-specific) factors.

Non-genomic Effects

It appears that aldosterone also acts, in some cells, via a membrane receptor which is more selective for aldosterone than the nuclear receptor. The activation of this receptor rapidly stimulates the activity of the Na/H antiporter, possibly via the inositol-3P pathway [80, 130, 135]. Hence, it could be that the hormone specificity of the mineralocorticoid action is mediated by the action of this membrane receptor in certain cells. Its activation could also have a synergistic and/or permissive effect on the nuclear MR action. However, no functional role has yet been attributed to this receptor in classical Na reabsorbing epithelia and no rapid, transcription-independent activation of the Na/H antiporter by aldosterone has been detected in A6 epithelia (V. Casavola and L. Guerra, *personal communication*).

Specificity Levels and the Na Transport Response

In view of the common, interconnected pathways and the possibility of multiple levels of specificity for mineralo- and glucocorticosteroid hormones and receptors discussed above, it is not surprising that different experimental systems and approaches have led to different conclusions concerning the role of these hormones and receptors in mediating the typical increase in Na reabsorption. Furthermore, the importance of inter-species differences in the role of aldosterone and of the MR is not clear. In general, it appears that mineralocorticoids can produce a major action in vivo in certain epithelia and in some cases also in cell culture systems at concentrations at which the GR is only marginally occupied [9, 25, 42, 76, 78, 79, 99, 111]. Hence, in these cases the effect is expected to be mediated by the MR. It has also clearly been demonstrated that the "protection" mechanism by 11β-OHSDH plays an important role in preventing the occupation of the MR and GR by cortisol in such epithelia (or corticosterone) [19, 42, 66, 79, 132]. However, it has been shown in other experimental systems that the typical response could also be triggered, or only triggered, by the occupation of the GR or that the occupation of this low affinity receptor by an agonist was necessary for a maximal response [48, 49, 68, 106, 134]. In some instances, it has been postulated that the MR and the GR could be involved in producing different phases of the hormonal response. However, recent and older experiments show that this hypothesis does not account for the actual complexity of the situation [48, 49]. Furthermore, there is a major concern about all studies addressing the question of the receptor-specificity using agonists and antagonists to respectively activate and block one and the other adrenal steroid receptor. Not only are the binding and agonist and/or antagonist properties of these compounds to some extent species-

specific, but, as suggested by the discussion above, their complex with a receptor might have differential agonist or antagonist properties depending on the gene and the context and nature of the HRE('s) and presence of other factors.

Despite the experimental limitations mentioned, it appears that the natriferic response observed in A6 cell cultures are mainly mediated by the GR or low affinity receptor although a MR or high affinity receptor is present [106, 134]. Similarly, the M-1 cell line which is derived from the cortical collecting duct of a mouse [114] also appears to respond to corticosteroids exclusively via the GR (B. Rossier and H.P. Gaeggeler, *personal communication*). This difference between immortalized cells and the epithelia of origin might be due to the lack, in cultured cells, of a (specificity-conferring) mechanism or factor necessary for the regulation of (a) mediator(s) of the Na transport response by the MR but not by the GR. In this regard, primary cultures of rat inner medullary collecting duct cells are an interesting experimental system [68]. In this case a natriferic response can be elicited via both receptors though with differences at the level of its time course and of the Na,K-ATPase induction. This underlines the existence of similarities and differences in the gene network regulated by the two receptors.

Adrenal-Steroid-Regulated Gene Products

Adrenal steroids have been shown on the one hand to directly (and indirectly) modulate the transcription of specific genes (*see above*) and on the other hand to induce changes in Na reabsorption which are dependent on ongoing transcription and translation [35, 45, 99]. How the transcriptional events lead to the physiological response is still not known. Two complementary approaches have been undertaken to fill this gap of understanding [125]. The first or "forwards" approach consists of starting at the level of transcriptional regulation, by identifying adrenal-steroid-regulated gene products in the target cells (generally called aldosterone-induced proteins: AIP's) which then can be tested for their role in the mediation of the Na transport response. The second or "backwards" approach consists of identifying the effectors of the physiological response and their regulators and then to test for their potential transcriptional induction. It has to be recalled that physiological studies indicate that the transport response is divided into different phases (*see below*). The expectation is that the induced/repressed gene products mediating the early response directly or indirectly regulate the activity of pre-existing effector proteins (Na channel, Na,K-ATPase, etc.), whereas the late response would be characterized by the increased synthesis of effector proteins (constitutive elements of the Na transport machinery).

From the Genes to the Function

Experiments belonging to the first (forwards) approach have been undertaken at the RNA and protein levels using progressively more specific techniques [45, 99, 115]. By two-dimensional polyacrylamide gel electrophoresis of metabolically labeled proteins, gene products induced or repressed in their synthesis by aldosterone have been visualized. In most experiments the analysis was performed several hours after the addition of the hormone, at a time at which downregulation, secondary and indirect effects are already complicating the situation. From such experiments performed on toad urinary bladder it is known that at least 15 out of 1,000 gene products are affected in their expression by a hormonal treatment (18 hr aldosterone 10^{-7} M) [117]. More recently it has been shown that one of the visualized regulated proteins (GP70) to which a monoclonal antibody has been raised was induced in its synthesis as early as 1 to 2 hr after hormone addition, and it appeared to associate with the epithelial Na channel complex purified by Benos et al. [13, 15, 115]. However, its nature and its potential role in the mediation of the Na transport response are not known.

The direct identification of cDNAs derived from regulated mRNAs has the great advantage of directly providing a molecular probe and potentially interesting sequence information. This type of approach has recently become easier and more efficient with the introduction of polymerase chain reaction (PCR) amplification steps in subtractive hybridization and differential screening protocols. An impressive example of the use of such a method is that of the study of the thyroid hormone induced tadpole tail resorption by the group of D. Brown [133]. Another straightforward technique is the mRNA differential display. It is based on the amplification by RT-PCR of the 3′ ends of poly(A^+) RNAs using a set of anchored oligo-dT primers in conjunction with arbitrary 10mers [70]. The comparison of the intensity of electrophoretically separated bands generated from mRNAs originating from differentially treated cells allows the identification and cloning of cDNA fragments derived from regulated mRNAs. A disadvantage of this technique is that the fragments mostly correspond to 3′ untranslated regions of the mRNAs, such that a further step of cDNA library screening is necessary to obtain the coding sequence. A problem of these techniques is that the representativity of the result might be biased by the preferential reverse transcription and amplification from certain mRNAs.

The mRNA differential display method, by allowing the visualization of signals (bands) corresponding to a large number of individual mRNAs, can generate valuable information on the overall impact of a hormonal treatment on gene expression since the fraction of mRNAs modulated by the treatment can be estimated.

The specificity of this action (e.g., comparison of mineralo- and glucocorticoids) and the time course of a response can also be readily analyzed.

Whichever technique is used for the identification of regulated mRNAs, a difficult task will be to test the involvement of the encoded proteins in the Na transport response. The growing number of published sequences is a help in this regard since it makes it more likely that the comparison of sequences from cloned cDNAs with those from data banks will identify homologous proteins from other systems or homologous sequence motifs found in other proteins. This type of information enables the researcher to make predictions about the function of the protein encoded by the newly identified cDNAs. In any case the demonstration that a gene product plays a role in the Na transport response will finally have to be made by manipulating its expression in a target epithelium and monitoring the impact on the hormonally regulated Na transport.

Using a conventional differential screening protocol of rat and chicken colon cDNA libraries, Garty has identified several clones corresponding to transcripts enhanced by a dexamethasone treatment or by a low salt diet, respectively (H. Garty, *personal communication*). Interestingly, some of these cDNAs code for oxidative phosphorylation enzymes encoded by the mitochondrial genome. The fact that enzymes implicated in the energy output of the mitochondria have been identified lends new support to the old hypothesis that aldosterone acts on Na transport by a pleiotropic action which includes the increase in ATP supply for the Na/K pump (*see for review* [45, 61, 98, 99]). It is surprising, however, that adrenal steroids induce an accumulation of mRNAs encoded by the mitochondrial genome. Possible explanations could be that the transcription rate of mitochondrial genes or the number of mitochondria is increased secondarily to the action of (an) aldosterone-regulated protein(s) encoded by the nuclear genome.

Using the technique of mRNA differential display PCR on mRNA of kidney CCD cells treated with aldosterone (10^{-7} M), the group of A. Náraj-Fejes-Tóth has identified several up- and downregulated bands [31]. As yet, none of the (short) cloned cDNAs appears to be homologous to sequences of the data bank.

Using the same approach to analyze the response to aldosterone (10^{-6} M) in A6 cells, we found that approximately 1% of the bands were reproducibly up- or downregulated after one hour of hormone treatment (B. Spindler and F. Verrey, *unpublished observation*).

From the Function to the Genes

The second or "backwards" approach for the identification of transcriptionally regulated mediator(s) is based on the knowledge of the effectors and their potential regulators and requires molecular tools to measure their expression.

Concerning the potential regulation of structural proteins participating in the Na transport, it has been shown so far that both major transport proteins involved in the regulated Na reabsorption, i.e., the epithelial Na channel (α,β,γENaC, originally cloned from rat distal colon and homologues of which are present in A6 cells and the toad bladder ([22, 23, 97] and B. Rossier, *personal communication*) and the Na,K-ATPase $\alpha 1$ and $\beta 1$ subunits are induced a few fold in amphibian tight epithelia at the mRNA level ([128, 129], B. Rossier and A. Puoti, *personal communication*). In the case of the Na,K-ATPase subunits the induction has been traced back to a very early transcriptional effect (twofold increase after a 15 min hormone treatment) while the actual accumulation of protein has been shown to take place during the late response [14, 124, 128]. It appears from preliminary results at the mRNA level that, like the induction of Na,K-ATPase subunits, the induction of Na channel subunits might play a role for the "late" transport response.

In some systems the activity of citrate synthase, which is part of the mitochondrial ATP synthesis machinery (*see also above*) appears to be upregulated by aldosterone. However, its rate of synthesis was not modified significantly in amphibian model epithelia [61, 98, 124]. It is interesting to mention here that a coordinate glucocorticoid-specific induction of Na,K-ATPase and mitochondrial oxydative enzymes has been described in the medullary thick ascending limb of the kidney of weaning rats [32]. This example shows that similar coordinated regulatory processes (*see also* cloning experiments from colon libraries mentioned above) might take place in different cells and states of development which may exhibit a different (cell specific) adrenal steroid specificity.

The only (preliminary) report concerning the induction/repression by adrenal steroids of regulatory proteins possibly involved in the mediation of the early Na transport response concerns the biosynthesis of a G-protein α-subunit [96]. Aldosterone (10^{-6} M) appeared to stimulate its synthesis in A6 cells. It will be interesting to investigate the time course, the level (transcriptional ?) and the role of this regulation. Besides this G protein, which is known to be implicated in the regulation of the Na channel and might play a role for the adrenal steroid action, no other candidate mediators have been tested so far.

In this context it is worth mentioning the case of the adenylate cyclase. Although it is not implicated in the mediation of the aldosterone effect, it plays an important role for aldosterone-induced transport, since it is part of a signaling pathway which produces a synergistic effect at the level of the Na channel activity and transepithelial Na reabsorption [105, 124]. It has been shown in rat

Table 1. Gene products regulated by adrenal steroids in tight epithelia

Protein	Epithelium	Regulated level investigated	Hormonal treatment (M)	Earliest change	Fractional change (time)	References
Na/K-ATPase						
α1 subunit	Toad bladder	Protein synthesis	Aldo (0.8×10^{-7})		2.8× (18 hr)	[50a]
	A6	Protein pool	Aldo (10^{-6})	5 hr	2.0× (5 day)	[14]
	A6	Protein synthesis	Aldo (10^{-7}, 3×10^{-7})	3 hr	1.7× (6 hr)	[82, 129]
	A6	mRNA pool	Aldo (3×10^{-7})	3 hr	2.0× (6 hr)	[128, 129]
	A6	Transcription	Aldo (3×10^{-7})	15 min	1.8× (45 min)	[128]
	CCD rabbit	Protein pool	Aldo *in vivo* (4×10^{-9})		3.4× (5–10 day)	[136]
β1 subunit	Toad bladder	Protein synthesis	Aldo (0.8×10^{-7})		2.4× (18 hr)	[50a]
	A6	Protein pool	Aldo (10^{-6})	5 hr	2.1× (5 day)	[14]
	A6	Protein synthesis	Aldo (10^{-7}, 3×10^{-7})	3 hr	2.0× (6 hr)	[82, 129]
	A6	mRNA pool	Aldo (3×10^{-7})	3 hr	4.0× (6 hr)	[128, 129]
	A6	Transcription	Aldo (3×10^{-7})	15 min	2.9× (45 min)	[128]
	CCD rabbit	Protein pool	Aldo *in vivo* (4×10^{-9})		3.6× (5–10 day)	[136]
Epithelial Na channel						
α-xENaC	A6	mRNA pool	Aldo (10^{-6})	3–20 hr	2.3× (20 hr)	A. Puoti, F. Verrey and B. Rossier, u.o.
β-xENaC	A6	mRNA pool	Aldo (10^{-6})	3–6 hr	2.6× (20 hr)	
γ-xENaC	A6	mRNA pool	Aldo (10^{-6})	1.5-3 hr	3.6× (20 hr)	
Cytochrome C oxydase I, II, III	Rat distal colon	mRNA pool	Dexamethasone injection		2.5× (3 day)	H. Garty, u.o.
NADH dehydrogenase I	Chicken colon	mRNA pool	Low salt diet		~3× (4 weeks)	H. Garty, u.o.
GP70	A6, toad bladder	Protein synthesis	Aldo (10^{-7})	1–2 hr	+++ (2–3 hr) (not quantitative)	[15]

u.o. stands for *unpublished observations.*

CCD and in A6 cells that the function of the adenylate cyclase is upregulated by aldosterone [36, 126]. Whether this effect corresponds to an increase in its synthesis, altered function or both is not known.

Adrenal-steroid-regulated gene products (or less general: aldosterone-induced proteins) are listed in the Table. Only those which have been shown to be induced at the level of the biochemical (immunoreactive) protein pool and/or upstream of it and which have been characterized to some extent or the identity of which is known are included. It is expected that this brief list will expand rapidly because of the availability of techniques for the rapid identification of regulated mRNAs. However, the establishment of links from the induced proteins to the physiological function will be required to allow an understanding of the mechanism(s) by which a transcriptional regulation leads to a change in transepithelial Na transport. This will not be an easy task, especially if gene products unrelated to a concerted action on Na transport are also induced. An interesting possibility is that activated adrenal steroid receptors modulate the transcription of other transcription factor(s) [59] which could, in turn, produce a secondary response (from a molecular point of view, *see below*) possibly involved in the late phases of the physiological hormone action.

Distinct Early and Late Responses to Adrenal Steroids in Tight Epithelia

Molecular and Physiological Cascades

Most experimental observations of hormone action discussed here are made upon switching acutely from a low hormone or control situation to a high hormone or test situation. Such a switch, though not very physiological, allows the observation of a synchronous physiological response and is necessary for the analysis of the underlying cascade of events. Indeed, in the case of the corticosteroid action on Na transport, two types of interconnected and arbitrarily dissociated cascades have to be considered: the "molecular" and the "physiological" cascades.

From a "molecular" point of view, the ligand-activated nuclear receptors are producing a so-called pri-

mary response which promotes the direct regulation of a particular set of genes, which have yet to be identified (*see above*). Products of these genes can modify the transcription rate of a second set of genes (secondary response) if they happen to be transcription factors or to regulate (directly or indirectly) other transcription factors. This cascade of events can also continue to produce a tertiary response and so forth.

From a "physiological" point of view of ion transport, the primary (functional) response is the initial change in ion transport and is the direct consequence of the "molecular" cascade mentioned above. These changes in ion transport will impact on cellular parameters such as intracellular ion concentrations, ATP/ADP ratio, etc. Thereby so-called indirect effects might be triggered which can again implicate regulatory mechanisms at the transcriptional level.

The intricateness of "molecular" responses and indirect "physiological" effects is typical for "late" events. An example is that of the increase in Na,K-ATPase synthesis in response to aldosterone. Indeed, its subunits are under early transcriptional control by adrenal steroids [124, 128] but since their synthesis is also controlled by the intracellular Na concentration [18, 95, 116, 141] the "physiological" changes in Na influx and/or extrusion induced by the hormonal treatment can interfere with the "molecular" hormone action. The fact that late effects are more subject to (feedback) regulation than early effects does not mean that they are physiologically less relevant. It simply appears that it is more complicated to establish the sequence of regulatory events leading to their development.

At this stage of the discussion, it is useful to remember that the complicated sequence of events discussed above concerns the cellular level only. The situation is by far more complicated in a whole organism where colinear and feedback regulatory events implicate a wealth of interdependent general and local physiological and hormonal parameters.

Early Response to Adrenal Steroids

In the toad bladder and A6 cells the electrophysiologically measurable functional (physiological) response to aldosterone (or dexamethasone) can be divided into three or four phases [14, 45, 46, 98, 99, 110, 124]. A schematic representation of the time course of functional responses is given in Fig. 2.

The first period is a lag phase which lasts approximately 20 (in mammalian systems) to 60 min from the addition of the hormone and during which the physiological parameters do not change. The second phase is referred to as an early response phase and is characterized by a progressive increase in transepithelial Na transport which appears to be generally proportional to the pre-existing transport activity (two- to fivefold increase) and which reaches a maximum within 2 to 4 hr after hormone addition. This effect is mediated mainly by an activation of pre-existing apical Na channels [47, 86]. However, although the apical influx of Na into the cell is generally the rate limiting step of transcellular transport, a coordinated *in situ* activation of the Na,K-ATPase has also been demonstrated which is independent of this Na influx [14, 89]. Furthermore, a basolaterally located K channel which could play a role for the recirculation of the potassium pumped into the cell by the Na,K-ATPase, is also activated in A6 and toad bladder cells during the early phase of aldosterone action [20, 56]. A model for the mechanism of hormone action for the early response phase is given in Fig. 3*A*.

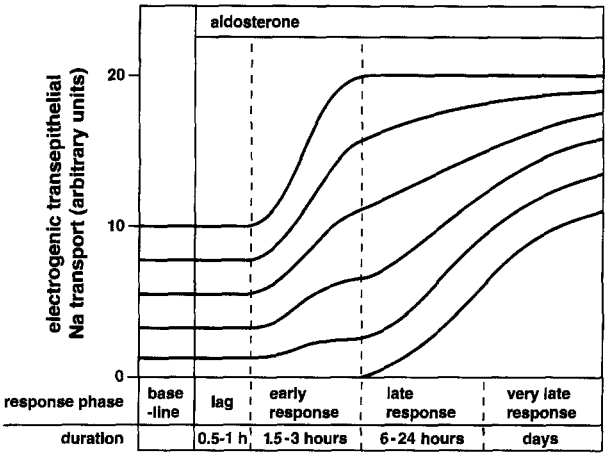

Fig. 2. Schematic time courses of adrenal steroid action on Na transport at various baseline transport rates. The idealized curves are based on experiments performed on A6 cell epithelia in this and other laboratories [14, 82, 89, 125, 126, 138]. It is suggested that the relative importance of the early and late responses of epithelia to the administration of aldosterone (10^{-7}–10^{-6} M) depends on the baseline expression level of the Na transport machinery which is reflected (in this figure) by the baseline transport level. The early response takes place after a 30–60 min lag period and is characterized by a two- to fivefold increase in Na reabsorption within 1.5 to 3 hr. The extent of the late response depends on the state of the epithelium prior to the hormone treatment. The maximal transport rates indicated are taken arbitrarily.

A patch clamp study on A6 cells re-exposed to aldosterone after a withdrawal period suggests that the mechanism by which Na channels are activated during the early response corresponds to a switch of pre-existing channels from a state of low open probability and short open times to one with a high open probability and long open times [65]. This interpretation is compatible with the observation that antidiuretic hormone, which is known to increase the number of active channels [73], acts synergistically with aldosterone at the level of the Na channels [105, 125]. However, it has to be men-

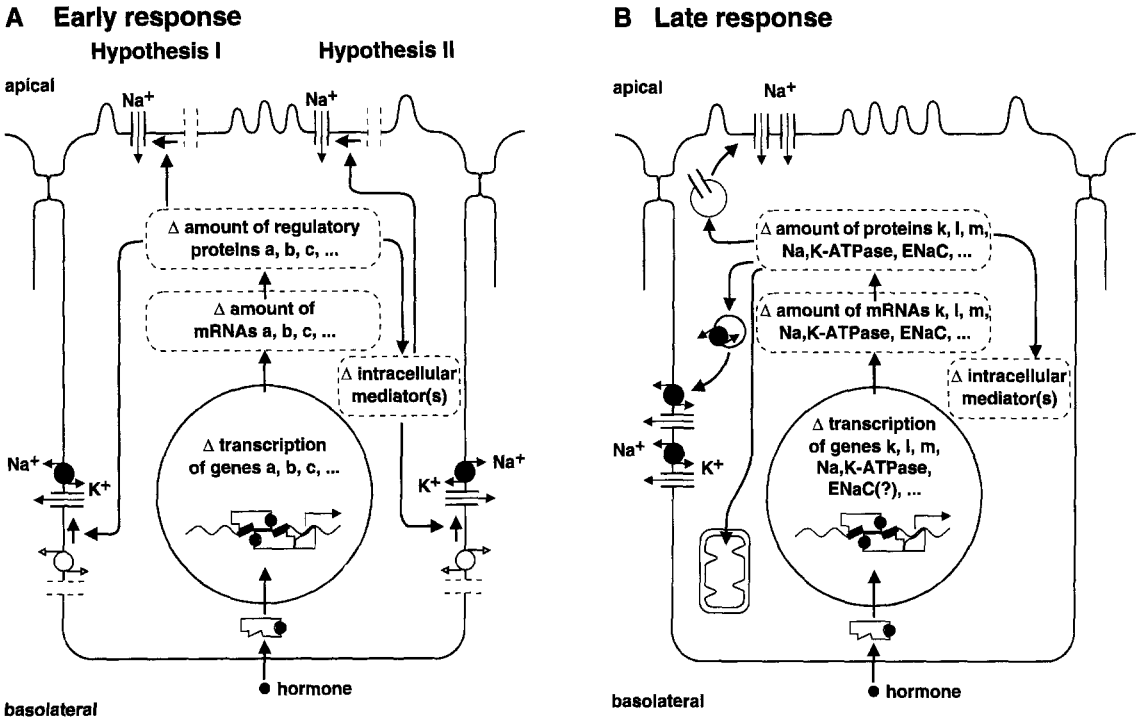

Fig. 3. Models for the early and late responses to aldosterone in tight epithelia. (*A*) Early response: the products of transcriptionally regulated genes activate *in situ* either directly (*Hypotheses I*) or indirectly (by a cascade of events, *Hypothesis II*) pre-existing apical Na channels and basolateral Na pumps (as well as K channels and possibly other effectors of the regulated Na transport). (*B*) Late response: the transcription of genes coding for elements of the Na transport machinery, such as the Na,K-ATPase α1 and β1 subunits, is increased as a primary effect (or for some genes eventually secondary effect) of the hormone-receptor complex such that the Na transport capacity of the cell is increased. The effect of the early response can persist during the late response.

tioned that in a patch clamp study on CCD's isolated from rats, no low open probability state of the Na channels was observed. Only changes in the channel number were visualized [87]. This difference with the A6 system was suggested to be quantitative rather than qualitative.

It is not clear whether or not the activation (or rather derepression) of the Na,K-ATPase, which has been measured in A6 cell epithelia as an increase in the rate of ouabain binding in K-free buffer, is due to a modification of the kinetics of active pumps or to an activation of previously silent pumps [14]. The mediator(s) of the early activation of both the channel and the pump has (have) not yet been identified. It might be that the same or different mediator(s) is (are) involved in these two processes. Several potential mechanisms for mediating this action at the level of the apical Na channel have been proposed, such as channel transmethylation or signaling cascades involving protein isoprenylation, G proteins, interactions with the cytoskeleton or Ca_i [90, 96, 103, 104, 109]. Basically, the activation of the transport proteins could be mediated directly by (an) induced (repressed) gene product(s) or by a cascade of events (Fig. 3*A*). Modification of the functional state of the transporters could be generated either by their covalent modification (i.e., by methylation or phosphorylation) or by noncovalent interactions. The latter could involve cytoskeletal elements, membrane components or soluble factors (including electrolytes). In this respect it is interesting to mention that the "early" activation of channels and pumps appears to depend on the integrity of epithelial and cellular structures indicating that either noncovalent or readily reversible covalent modification of the transporters is involved ([5, 14], J. Beron and F. Verrey, *unpublished observations*).

The fact that during the early response the increase in Na transport is proportional to the pre-existing one (in amphibian model epithelia, *see* Fig. 2) could be explained by the existence, in unstimulated cells, of a small Na influx via pre-existing apical channels in a low open probability state. This would also explain why the initial increase in transepithelial conductance, which is expected to be proportional to the number of channels activated during the early response and which was originally considered as the hallmark of the early response, is also generally proportional to the pre-existing Na transport (M.-P. Paccolat, doctoral thesis, University of Lausanne, 1987). In agreement with this, no functional response is observed before the beginning of the late response in the absence of pre-existing transport [82]. Finally, the state of activation (or derepression) induced

during the early response could last as long as the hormone is present or, alternatively, it could be downregulated in the course of the (very) late response when the transport capacity is increased (*see below*) [14]. It can therefore be expected that the regulatory factor(s) which mediate an early physiological response by activating pre-existing effectors (channels and pumps) are also induced in cells which do not express enough baseline effectors (and Na transport) to show a functional early response. In such a case new transporters produced in the course of the late response would first appear in the activated state.

Late Response to Adrenal Steroids

The third phase is the late response which was originally described in the toad bladder as a further increase in Na transport starting approximately 3 hr after hormone addition and which was not paralleled by an increase in transepithelial conductance [98, 110]. However, in target epithelia in which the baseline electrogenic Na transport is lower than in the toad bladder the increase in Na transport during the late response can be more important and accompanied by an increase in transepithelial conductance [125].

This late increase in Na transport could be mediated by an increase in the number of Na channels, possibly compensated to some extent by a concomitant increase in (para)cellular resistance [56], or, alternatively, by an increase in the driving force for Na transport. Results from two types of experiments support the first possibility without excluding a role for the second one. First, mRNA isolated from chronically stimulated epithelia was shown (with one exception [85]) to induce a higher level of Na transport in the oocyte expression system than mRNA isolated from control epithelia [4, 6, 55]. Second, and more specifically, preliminary experiments with the cDNAs encoding the three recently cloned subunits of the epithelial Na channel show that their mRNA level is increased by an aldosterone treatment (*see* above and the Table) ([23], B. Rossier and A. Puoti, *personal communication*). That the Na,K-ATPase synthesis and pool are increased during the late phase has also been mentioned above [14, 128, 129]. These observations are in agreement with the notion that the late effect is, at least to some extent, due to an increase in the amount of effector proteins (*see* model in Fig. 3*B*). It has to be stressed that the increase in transporters is considered as a late effect based on the time point of its impact on the functional response and not on the initial molecular events which lead to their late accumulation. Interestingly, the late phase of adrenal steroid action has been shown to be specifically inhibited (in toad bladder) by thyroid hormone or Na butyrate [50, 118] and by brefeldin A (0.1 μg/ml in A6 cells) [26]. This confirms that the late response depends on the regulation of a (set of) gene(s) which is not required for the mediation of the early response, and, furthermore, the effect of brefeldin A suggests that the (synthesis and) delivery of membrane proteins to the cell surface is only required for the late response.

Long-term exposure of epithelia to adrenal steroid hormone can produce a further increase in Na reabsorption, as for instance in A6 cell epithelia (approximately 30% increase from 1 to 5 days of hormonal treatment [14]). During this "very late" phase of hormone action [99] morphogenetic "adaptative" effects can take place such as the increase in basolateral cell surface area described in CCD cells after in vivo treatment [63, 131]. It is not clear (and difficult to address experimentally) to what extent these effects are due to a direct "anabolic" effect of the hormone and/or to indirect effects mediated by the functional changes in transport and/or to "collateral" actions of the hormone treatment (*see e.g.*, [137]). In contrast to the studies mentioned above, Palmer et al., [84] did not find an increase of the membrane capacitance (indicative of membrane surface area) in cortical collecting duct principal cells of rats treated in the long term with low salt diet or aldosterone. Furthermore, there was no increase in the maximal Na pump current. These contrasting results obtained in different studies on long-term effects is not surprising in view of the complex regulatory networks involved. For instance, the lack of measurable effect mentioned above corresponds to the observation of Wiener et al. [137] who showed that a low sodium diet induces only a transient increase in Na,K-ATPase mRNA and pump sites in rabbit distal colon. Interestingly, this late escape from the effect of chronic hyperaldosteronism was shown to be mediated by a decrease in thyroid hormone levels.

The question of the hormone and receptor specificity of the adrenal steroid action on Na transport and the fact that there are significant differences in this regard between experimental systems has been discussed above.

Implications for the Mammalian Tight Epithelia

The temporal organization of the natriferic response is very similar in different amphibian model epithelia analyzed in vitro such as the A6 cells and the toad bladder but it is not clear how these responses relate to the situation observed in mammals. It has to be stressed in advance that all comparisons made here and elsewhere between results obtained with different systems and in different conditions have to be interpreted with caution since the regulated parameters are themselves influenced by the experimental procedure. For instance, it appears reasonable to suspect that the functional state of transporters belonging to the transepithelial Na reabsorption machinery depends on the isolation procedure and in

vitro incubation conditions of tested epithelia. For example, the early stimulation of apical Na channels, in contrast to the late stimulation, is not maintained in vesicles isolated from toad bladders [5].

The comparisons are restricted here to the time course of the response to aldosterone (or DOCA) studied in isolated mammalian kidney cortical ducts or distal colons, stimulated in vivo or in vitro. In cortical collecting ducts the baseline transport has generally been reported to be low when they are isolated from adrenalectomized animals or even from adrenal-intact animals. The response to in vivo injected mineralocorticoid hormone appears after a lag period of two to three hours (adrenalectomized animals) or days (adrenal-intact animals) [33, 81, 87, 99]. One possible interpretation is that these epithelia lack a functional reserve of pre-existing effectors such that, in analogy to amphibian model epithelia with low baseline transport, little or no "early" increase in Na transport can take place. If early events equivalent to those which mediate the early response in amphibian model epithelia nevertheless take place, newly synthesized transporters appearing during the delayed response (which would correspond to the late and/or very late response) would immediately be activated. An alternative interpretation is that, though the tubules of adrenalectomized and adrenal-intact animals have sufficient effectors to produce an early response in vivo, the procedure of tubule isolation and in vitro incubation would *per se* block the early activation such that only the late type of effect would be preserved in vitro.

However, in contrast to the slow effects on Na transport discussed above, in vitro treatment of isolated cortical collecting tubules with aldosterone has been shown to produce, after a lag period of approximately 30 min, an activation of the Na,K-ATPase function [41] or an increase in Na,K-ATPase activity (with a permissive role for triiodothyronine) [8]. It has been postulated that such early effects would not take place in vivo because of an inhibitory action of systemic or local factors. However, again it could be that the early stimulatory effects on Na transport induced in vivo are prevented or reversed by the tubule isolation and incubation procedure. Indeed, it appears that the incubation conditions are critical for the channel function since so far only early Na channel-independent effects on the Na,K-ATPase have been documented in this system.

In distal colon, baseline amiloride-sensitive, electrogenic Na transport is very low and it is only upon stimulation by aldosterone that this type of transport consistently appears [40, 53]. In this case no early increase due to the activation of pre-existing transporters can be expected and the observed increase, after a lag of 1.5–3 hr, probably corresponds to a "late" response, the time course of the response being expected to be faster at 37°C than at the colder temperatures used for amphibian epithelia [40, 53, 62].

Conclusions

The model for the adrenal steroid action on Na transport in tight epithelia as depicted in Fig. 3*A* and *B* dissociates two phases: an early phase during which the pre-existing Na transport machinery is activated and a late phase during which the transport capacity of the machinery is increased. These two sequential phases have been distinguished based on differences in functional aspects of the induced transport, on selective effects of agents interfering with transcriptional regulation and on a correlation of the late response phase with an increase in transport protein synthesis and expression [26, 45, 46, 98, 99, 124]. These observations suggest that a bimodal stimulation of Na transport could involve two different gene networks which are directly (in the physiological meaning) and independently stimulated by the action of the hormone-receptor complex and the following "molecular" cascades (*see* section Molecular and Physiological Cascades). The relatively clear temporal dissociation of the responses found in experimental situations is probably the consequence of inherent properties of the two networks. Indeed, to generate rapid functional changes, the genes involved in the early response must encode products which have relatively short half-lifes at the mRNA and protein levels. In contrast, the constitutive elements of the Na transport machinery that are increased during the late phase of adrenal steroid action have, as shown for the Na,K-ATPase [82], relatively long half-lifes. Consequently, even though changes in transcription may take place early in the course of the hormonal treatment, they impact on protein synthesis and pools only slowly and after a substantial lag period.

On the one hand, ongoing research will soon provide more information on the nature, time course and hormone/receptor specificity of adrenal-steroid-regulated genes. On the other hand, the availability of new technical and molecular tools to study the proteins of the Na transport machinery greatly increases the possibilities for studying its regulation by adrenal steroids. Consequently, it will be a fascinating challenge to relate the data emerging from both approaches, and it appears that only a combination of methods and tools will allow to progressively fill the gap of understanding which still lies between the transcriptional effects and the transport regulation.

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References

1. Adler, A.J., Danielsen, M., Robins, D.M. 1992. Androgen-specific gene activation via a consensus glucocorticoid response

element is determined by interaction with nonreceptor factors. *Proc. Natl. Acad. Sci. USA* **89:**11660–11663
2. Allan, G.F., Tsai, S.Y., Tsai, M.J., Omalley, B.W. 1992. Ligand-dependent conformational changes in the progesterone receptor are necessary for events that follow DNA binding. *Proc. Natl. Acad. Sci. USA* **89:**11750–11754
3. Arriza, J.L., Simerly, R.B., Swanson, L.W., Evans, R.M. 1988. The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. *Neuron* **1:**887–900
4. Asher, C., Eren, R., Kahn, L., Yeger, O., Garty, H. 1992. Expression of the amiloride-blockable Na^+ channel by RNA from control versus aldosterone-stimulated tissue. *J. Biol. Chem.* **267:**16061–16065
5. Asher, C., Garty, H. 1988. Aldosterone increases the apical Na^+ permeability of toad bladder by two different mechanisms. *Proc. Natl. Acad. Sci. USA* **85:**7413–7417
6. Asher, C., Singer, D., Eren, R., Yeger, O., Dascal, N., Garty, H. 1992. NaCl-dependent expression of amiloride-blockable Na^+ channel in *Xenopus* oocytes. *Am. J. Physiol.* **262:**G244–G298
7. Barlet-Bas, C., Cheval, L., Feraille, E., Marsy, S., Doucet, A. 1991. Regulation of tubular Na-K-ATPase. *In:* Nephrology. M. Hatano, editor. pp. 419–434. Springer-Verlag, Tokyo
8. Barlet-Bas, C., Khadoury, C., Marsy, S., Doucet, A. 1988. Sodium-independent in vitro induction of Na^+,K^+-ATPase by aldosterone in renal target cells: Permissive effect of triiodothyronine. *Proc. Natl. Acad. Sci. USA* **85:**1707–1711
9. Bastl, C.P., Hayslett, J.P. 1992. The cellular action of aldosterone in target epithelia. *Kidney Int.* **42:**250–264
10. Bastl, C.P., Schulman, G., Cragoe, E.J. 1992. Glucocorticoids inhibit colonic aldosterone-induced conductive Na^+ absorption in adrenalectomized rat. *Am. J. Physiol.* **263:**F443–F452
11. Baumann, H., Paulsen, K., Kovacs, H., Berglund, H., Wright, A.P.H., Gustafsson, J.A., Hard, T. 1993. Refined solution structure of the glucocorticoid receptor DNA-binding domain. *Biochemistry* **32:**13463–13471
12. Beekman, J.M., Allan, G.F., Tsai, S.Y., Tsai, M.J., O'Malley, B.W. 1993. Transcriptional activation by the estrogen receptor requires a conformational change in the ligand binding domain. *Mol. Endocrinol.* **7:**1266–1274
13. Benos, D.J., Saccomani, G., Brenner, B.M., Sariban-Sohraby, S. 1986. Purification and characterization of the amiloride-sensitive sodium channel from A6 cultured cells and bovine renal papilla. *Proc. Natl. Acad. Sci. USA* **83:**8525–8529
14. Beron, J., Verrey, F. 1994. Aldosterone induces early activation and late accumulation of Na,K-ATPase at surface of A6 cells. *Am. J. Physiol.* **266:**C1728–C1290
15. Blazer-Yost, B.L., Fesseha, Y., Cox, M. 1992. Aldosterone-mediated Na^+ transport in renal epithelia—Time-course of induction of a potential regulatory component of the conductive Na^+ channel. *Biochem. Int.* **26:**887–897
16. Bocquel, M.T., Kumar, V., Stricker, C., Chambon, P., Gronenmeyer, H. 1989. The contribution of the N- and C-terminal regions of steroid receptors to activation of the transcription is both receptor and cell specific. *Nucl. Acids Res.* **17:**2581–2595
17. Bourgeois, S., Gruol, D.J., Newby, R.F., Rajah, F.M. 1993. Expression of an MDR gene is associated with a new form of resistance to dexamethasone-induced apoptosis. *Mol. Endocrinol.* **7:**840–851
18. Bowen, J.D., McDonough, A.A. 1987. Pretranslational regulation of Na^+-K^+-ATPase in cultured canine kidney cells by low K. *Am. J. Physiol.* **252:**C179–C189
19. Brem, A.S., Morris, D.J. 1993. Interactions between glucocorticoids and mineralocorticoids in the regulation of renal electrolyte transport. *Mol. Cell. Endocrinol.* **97:**C1–C5
20. Broillet, M.C., Berger, A., Horisberger, J.D. 1993. Early effect of aldosterone on the basolateral potassium conductance of A6 cells. *Pfluegers Arch.* **424:**91–93
21. Burns, K., Duggan, B., Atkinson, E.A., Famulski, K.S., Nemer, M., Bleackley, R.C., Michalak, M. 1994. Modulation of gene expression by calreticulin binding to the glucocorticoid receptor. *Nature* **367:**476–480
22. Canessa, C.M., Horisberger, J.D., Rossier, B.C. 1993. Epithelial sodium channel related to proteins involved in neurodegeneration. *Nature* **361:**467–470
23. Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D., Rossier, B.C. 1994. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature* **367:**463–467
24. Chuard, F., Durand, J. 1992. Coupling between the intracellular pH and the active transport of sodium in an epithelial cell line from *Xenopus laevis*. *Comp. Biochem. Physiol.* **102:**7–14
25. Clore, J., Schoolwerth, A., Watlington, C.O. 1992. When is cortisol a mineralocorticoid. *Kidney Int.* **42:**1297–1308
26. Coupaye-Gerard, B., Kim, H.J., Singh, A., Blazer-Yost, B.L. 1994. Differential effects of brefeldin-a on hormonally regulated Na^+ transport in a model renal epithelial cell line. *Biochim. Biophys. Acta—Biomembr.* **1190:**449–456
27. Dahlman-Wright, K., Almlof, T., McEwan, I.J., Gustafsson, J.A., Wright, A.P.H. 1994. Delineation of a small region within the major transactivation domain of the human glucocorticoid receptor that mediates transactivation of gene expression. *Proc. Natl. Acad. Sci. USA* **91:**1619–1623
28. Dauvois, S., White, R., Parker, M.G. 1993. The antiestrogen ICI 182780 disrupts estrogen receptor nucleocytoplasmic shuttling. *J. Cell Sci.* **106:**1377–1388
29. Dedhar, S., Rennie, P.S., Shago, M., Hagesteijn, C.Y.L., Yang, H.L., Filmus, J., Hawley, R.G., Bruchovsky, N., Cheng, H., Matusik, R.J., Giguere, V. 1994. Inhibition of nuclear hormone receptor activity by calreticulin. *Nature* **367:**480–483
30. DeKloet, E.R., Sutanto, W., Vandenberg, D.T.W.M., Carey, M.P., Vanhaarst, A.D., Hornsby, C.D., Meijer, O.C., Rots, N.Y., Oitzl, M.S. 1993. Brain mineralocorticoid receptor diversity—functional implications. *J. Steroid Biochem. Mol. Biol.* **47:**183–190
31. Denault, D., Rusvai, E., Chen, W.R., Fejes-Tóth, G., Náray-Fejes-Tóth, A. 1993. Aldosterone-induced proteins and mRNAs in cortical collecting duct (CCD) cells. *J. Am. Soc. Nephrol.* **4:**437 (*Abstr.*)
32. Djouadi, F., Wijkhuisen, A., Bastin, J. 1992. Coordinate development of oxidative enzymes and Na-K-ATPase in thick ascending limb—role of corticosteroids. *Am. J. Physiol.* **263:**F237–F242
33. Doucet, A., Barlet-Bas, C. 1989. Involvement of Na^+,K^+-ATPase in antinatriuretic action of mineralocorticoids in mammalian kidney. *Curr. Top. Membr. Transp.* **34:**185–208
34. Duncan, R.L., Grogan, W.M., Kramer, L.B., Watlington, C.O. 1988. Corticosterone's metabolite is an agonist for Na^+ transport stimulation in A6 cells. *Am. J. Physiol.* **255:**F736–F748
35. Edelman, I.S., Bogoroch, R., Porter, G.A. 1963. On the mechanism of action of aldosterone on sodium transport: the role of protein synthesis. *Proc. Natl. Acad. Sci. USA* **50:**1169–1177
36. ElMernissi, G., Barlet-Bas, C., Khadouri, C., Cheval, L., Marsy, S., Doucet, A. 1993. Short-term effect of aldosterone on vasopressin-sensitive adenylate cyclase in rat collecting tubule. *Am. J. Physiol.* **264:**F821–F826
37. Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* **240:**889–895
38. Falvey, E., Schibler, U. 1991. How are the regulators regulated? *FASEB J.* **5:**309–314

39. Farman, N., Bonvalet, J.P., Seckl, J.R. 1994. Aldosterone selectively increases Na^+-K^+-ATPase alpha(3)-subunit mRNA expression in rat hippocampus. *Am. J. Physiol.* **266:**C423–C428
40. Fromm, M., Schulzke, J.D., Hegel, U. 1993. Control of electrogenic Na^+ absorption in rat late distal colon by nanomolar aldosterone added in vitro. *Am. J. Physiol.* **264:**E68–E73
41. Fujii, Y., Takemoto, F., Katz, A.I. 1990. Early effects of aldosterone on Na-K pump in rat cortical collecting tubules. *Am. J. Physiol.* **259:**F40–F45
42. Funder, J.W. 1993. Aldosterone action. *Annu. Rev. Physiol.* **55:**115–130
43. Funder, J.W. 1993. Mineralocorticoids, glucocorticoids, receptors and response elements. *Science* **259:**1132–1133
44. Gaeggeler, H.P., Duperrex, H., Hautier, S., Rossier, B.C. 1993. Corticosterone induces 11beta-HSD and mineralocorticoid specificity in an amphibian urinary bladder cell line. *Am. J. Physiol.* **264:**C317–C322
45. Garty, H. 1986. Mechanism of aldosterone action in tight epithelia. *J. Membrane Biol.* **90:**193–205
46. Garty, H. 1994. Molecular properties of epithelial, amiloride-blockable Na^+ channels. *FASEB J.* **8:**522–528
47. Garty, H., Edelman, I.S. 1983. Amiloride-sensitive trypsinisation of apical sodium channels. *J. Gen. Physiol.* **81:**785–803
48. Garty, H., Peterson-Yantorno, K., Asher, C., Civan, M.M. 1994. Effects of corticoid agonists and antagonists on apical Na^+ permeability of toad urinary bladder. *Am. J. Physiol.* **266:**F108–F116
49. Geering, K., Claire, M., Gaeggeler, H.P., Rossier, B.C. 1985. Receptor occupancy vs. induction of Na^+-K^+-ATPase and Na^+ transport by aldosterone. *Am. J. Physiol.* **248:**C102–C108
50. Geering, K., Gaeggeler, H.P., Rossier, B.C. 1984. Effects of thyromimetic drugs on aldosterone-dependent sodium transport in the toad bladder. *J. Membrane Biol.* **77:**15–23

50a. Geering, K., Girardet, M., Bron, C., Kraehenbuhl, J.P., Rossier, B.C. 1982. Hormonal regulation of (Na^+,K^+)-ATPase biosynthesis in the toad bladder. *J. Biol. Chem.* **257:**10388–10343

51. Guiochon-Mantel, A., Milgrom, E. 1993. Cytoplasmic nuclear trafficking of steroid hormone receptors. *Trends Endocrinol. Metab.* **4:**322–328
52. Guyton, A.C. 1991. Blood pressure control—special role of the kidneys and body fluids. *Science* **252:**1813–1816
53. Halm, D.R., Troutman-Halm, S. 1994. Aldosterone stimulates K secretion prior to onset of Na absorption in guinea pig distal colon. *Am. J. Physiol.* **266:**C552–C558
54. Harvey, B.J., Thomas, S.R., Ehrenfeld, J. 1988. Intracellular pH controls cell membrane Na^+ and K^+ conductances and transport in frog skin epithelium. *J. Gen. Physiol.* **92:**767–791
55. Hinton, C.F., Eaton, D.C. 1989. Expression of amiloride-blockable sodium channels in *Xenopus* oocytes. *Am. J. Physiol.* **257:**C825–C829
56. Horisberger, J.D. 1992. Early effects of aldosterone on apical and basolateral membrane conductances of TBM cells. *Am. J. Physiol.* **263:**C384–C388
57. Hsu, S.C., Qi, M., Defranco, D.B. 1992. Cell cycle regulation of glucocorticoid receptor function. *EMBO J.* **11:**3457–3468
58. Hutchison, K.A., Dittmar, K.D., Czar, M.J., Pratt, W.B. 1994. Proof that hsp70 is required for assembly of the glucocorticoid receptor into a heterocomplex with hsp90. *J. Biol. Chem.* **269:**5043–5049
59. Inoue, S., Orimo, A., Hosoi, T., Kondo, S., Toyoshima, H., Kondo, T., Ikegami, A., Ouchi, Y., Orimo, H., Muramatsu, M. 1993. Genomic binding-site cloning reveals an estrogen-responsive gene that encodes a RING finger protein. *Proc. Natl. Acad. Sci. USA* **90:**11117–11121
60. Ivarie, R.D., Baxter, J.D., Morris, J.A. 1981. Interaction of thyroid and glucocorticoid hormones in rat pituitary tumor cells. *J. Biol. Chem.* **256:**4520–4528
61. Johnson, J.P. 1992. Cellular mechanisms of action of mineralocorticoid hormones. *Pharmacol. Ther.* **53:**1–29
62. Jorkasky, D., Cox, M., Feldman, G.M. 1985. Differential effects of corticosteroids on Na^+ transport in rat distal colon in vitro. *A. J. Physiol.* **248:**G424–G431
63. Kaissling, B., Le Hir, M. 1991. Aldosterone: influence on distal tubule cell structure. *In:* Aldosterone: Fundamental Aspects, Colloque INSERM Vol 215. J.-P. Bonvalet, N. Farman, M. Lombès, and M.E. Rafestin-Oblin, editors. pp. 175–185. John Libbey Eurotext, Paris
64. Kang, K.I., Devin, J., Cadepond, F., Jibard, N., Guiochonmantel, A., Baulieu, E.E., Catelli, M.G. 1994. In vivo functional protein protein interaction–nuclear targeted hsp90 shifts cytoplasmic steroid receptor mutants into the nucleus. *Proc. Natl. Acad. Sci. USA* **91:**340–344
65. Kemendy, A.E., Kleyman, T.R., Eaton, D.C. 1992. Aldosterone alters the open probability of amiloride-blockable sodium channels in A6 epithelia. *Am. J. Physiol.* **263:**C825–C837
66. Kenouch, S., AlFaidy, N., Bonvalet, J.P., Farman, N. 1994. Expression of 11 beta-OHSD along the nephron of mammals and humans. *Steroids* **59:**100–104
67. Lanz, R.B., Hug, M., Gola, M., Tallone, T., Wieland, S., Rusconi, S. 1994. Active, interactive, and inactive steroid receptor mutants. *Steroids* **59:**148–152
68. Laplace, J.R., Husted, R.F., Stokes, J.B. 1992. 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
69. Legoff, P., Montano, M.R., Schodin, D.J., Katzenellenbogen, B.S. 1994. Phosphorylation of the human estrogen receptor—identification of hormone-regulated sites and examination of their influence on transcriptional activity. *J. Biol. Chem.* **269:**4458–4466
70. Liang, P., Pardee, A.B. 1992. Differential display of eukariotic messenger RNA by means of the polymerase chain reaction. *Science* **257:**967–971
71. Lombès, M., Oblin, M.E., Gasc, J.M., Baulieu, E.E., Farman, N., Bonvalet, J.P. 1992. Immunohistochemical and biochemical evidence for a cardiovascular mineralocorticoid receptor. *Circ. Res.* **71:**503–510
72. Luisi, B.F., Xu, W.X., Otwinowski, Z., Freedman, L.P., Yamamoto, K.R., Sigler, P.B. 1991. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature* **352:**497–505
73. Marunaka, Y., Eaton, D.C. 1991. Effects of vasopressin and cAMP on single amiloride-blockable Na channels. *Am. J. Physiol.* **260:**C1071–C1084
74. Marver, D., Stewart, J., Funder, J., Feldman, D., Edelman, I.S. 1974. Renal aldosterone receptors: studies with [3H]aldosterone and the antimineralocorticoid [3H]spironolactone (SC-26304). *Proc. Natl. Acad. Sci. USA* **71:**1431–1435
75. Meyer, M.E., Pornon, A.J., Ji, J., Bocquel, M.T., Chambon, P., Gronemeyer, H. 1990. Agonistic and antagonistic activities of RU486 on the functions of the human progesterone receptor. *EMBO J.* **9:**3923–3932
76. Morris, D.J., Souness, G.W. 1992. Protective and specificity-conferring mechanisms of mineralocorticoid action. *Am. J. Physiol.* **263:**F759–F768
77. Muchardt, C., Yaniv, M. 1993. A human homologue of saccharomyces-cerevisiae SNF2/SWI2 and drosophila-brm genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J.* **12:**4279–4290

78. Náray-Fejes-Tóth, A., Fejes-Tóth, G. 1990. Glucocorticoid receptors mediate mineralocorticoid-like effects in cultured collecting duct cells. *Am. J. Physiol.* **259:**F672–F678
79. Náray-Fejes-Tóth, A., Rusvai, E., Fejes-Tóth, G. 1994. Mineralocorticoid receptors and 11 beta-steroid dehydrogenase activity in renal principal and intercalated cells. *Am. J. Physiol.* **266:**F76–F80
80. Oberleithner, H., Vogel, U., Kersting, U., Steigner, W. 1990. Madin-Darby canine kidney cells. II. Aldosterone stimulates Na^+/H^+ and Cl^-/HCO_3^- exchange. *Pfluegers Arch.* **416:**533–539
81. O'Neil, R.G. 1989. Modulation of Na^+,K^+-ATPase expression in renal collecting duct. *Curr. Top. Membr. Transp.* **34:**209–228
82. Paccolat, M.P., Geering, K., Gaeggeler, H.P., Rossier, B.C. 1987. Aldosterone regulation of Na^+ transport and Na^+-K^+-ATPase in A6 cells: role of growth conditions. *Am. J. Physiol.* **252:**C468–C476
83. Palmer, L.G. 1992. Epithelial Na channels—Function and diversity. *Annu. Rev. Physiol.* **43:**51–66
84. Palmer, L.G., Antonian, L., Frindt, G. 1993. Regulation of the Na-K pump of the rat cortical collecting tubule by aldosterone. *J. Gen. Physiol.* **102:**43–57
85. Palmer, L.G., Corthesy-Theulaz, I., Gaeggeler, H.P., Kraehenbuhl, J.P., Rossier, B.C. 1990. Expression of epithelial Na channels in Xenopus oocytes. *J. Gen. Physiol.* **96:**23–46
86. Palmer, L.G., Edelman, I.S. 1981. Control of apical sodium permeability in the toad urinary bladder by aldosterone. *Ann. NY Acad. Sci.* **372:**1–14
87. Pácha, J., Frindt, G., Antonian, L., Silver, R.B., Palmer, L.G. 1993. Regulation of Na channels of the rat cortical collecting tubule by aldosterone. *J. Gen. Physiol.* **102:**25–42
88. Pearce, D., Yamamoto, K.R. 1993. Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science* **259:**1161–1165
89. Pellanda, A.M., Gaeggeler, H.P., Horisberger, J.D., Rossier, B.C. 1992. Sodium-independent effect of aldosterone on initial rate of ouabain binding in A6 cells. *Am. J. Physiol.* **262:**C899–C906
90. Petzel, D., Ganz, M.B., Nestler, E.J., Lewis, J.J., Goldenring, J., Akcicek, F., Hayslett, J.P. 1992. Correlates of aldosterone-induced increases in $Ca\text{-}_i^{(2+)}$ and Isc suggest that $Ca\text{-}_i^{(2+)}$ is the second messenger for stimulation of apical membrane conductance. *J. Clin. Invest.* **89:**150–156
91. Power, R.F., Mani, S.K., Codina, J., Conneely, O.M., Omalley, B.W. 1991. Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* **254:**1636–1639
92. Pratt, W.B. 1993. The role of heat shock proteins in regulating the function, folding, and trafficking of the glucocorticoid receptor. *J. Biol. Chem.* **268:**21455–21458
93. Rafestin-Oblin, M.E., Lombès, M., Couette, B., Baulieu, E.E. 1992. Difference between aldosterone and its antagonists in binding kinetics and ligand-induced hsp90 release from mineralocorticosteroid receptor. *J. Steroid. Biochem. Mol. Biol.* **41:**815–821
94. Ray, A., Prefontaine, K.E. 1994. Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappa B and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* **91:**752–756
95. Rayson, B.M. 1991. $[Ca^{2+}]_i$ regulates transcription rate of the Na^+/K^+-ATPase-alpha1 subunit. *J. Biol. Chem.* **266:**21335–21338
96. Rokaw, M.D., Palevsky, P.M., Johnson, J.P. 1993. Aldosterone (aldo) stimulates protein lipidation and G-protein (GP) synthesis in A6 cells. *J. Am. Soc. Nephrol.* **4:**446 (*Abstr.*)
97. Rossier, B.C., Canessa, C.M., Schild, L., Horisberger, J.D. 1994. Epithelial sodium channels. *Curr. Opinion Nephrol. Hypertension* **3:**487–496
98. Rossier, B.C., Paccolat, M.-P., Verrey, F., Kraehenbuhl, J.P., Geering, K. 1985. Mechanism of action of aldosterone: A pleiotropic response. *In:* Hormones and Cell Regulation Vol. 9, J.E. Dumont, B. Hamprecht and J. Nunez, editors. pp. 209–225. INSERM, Paris
99. Rossier, B.C., Palmer, L.G. 1992. Mechanisms of aldosterone action on sodium and potassium transport. *In:* The Kidney: Physiology and Pathophysiology (2nd ed.). D.W. Seldin and G. Giebisch, editors. pp. 1373–1405. Raven, New York
100. Rupprecht, R., Arriza, J.L., Spengler, D., Reul, J.M.H.M., Evans, R.M., Holsboer, F., Damm, K. 1993. Transactivation and synergistic properties of the mineralocorticoid receptor—relationship to the glucocorticoid receptor. *Mol. Endocrinol.* **7:**597–603
101. Rupprecht, R., Reul, J.M.H.M., Vansteensel, B., Spengler, D., Soder, M., Berning, B., Holsboer, F., Damm, K. 1993. Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands. *Eur. J. Pharmacol.* **247:**145–154
102. Sabatini, S., Hartsell, A., Meyer, M., Kurtzman, N.A., Hierholzer, K. 1993. Corticosterone metabolism and membrane transport. *Mineral Electrolyte Metab.* **19:**343–350
103. Sariban-Sohraby, S., Burg, M., Wiesmann, W.P., Chiang, P.K., Johnson, J.P. 1984. Methylation increases sodium transport into A6 apical membrane vesicles: possible mode of action of aldosterone action. *Science* **225:**745–746
104. Sariban-Sohraby, S., Fisher, R.S., Abramow, M. 1993. Aldosterone-induced and GTP-stimulated methylation of a 90-kDa polypeptide in the apical membrane of A6 epithelia. *J. Biol. Chem.* **268:**26613–26617
105. Schafer, J.A., Hawk, C.T. 1992. Regulation of Na^+ channels in the cortical collecting duct by AVP and mineralocorticoids. *Kidney Int.* **41:**255–268
106. Schmidt, T.J., Husted, R.F., Stokes, J.B. 1993. Steroid hormone stimulation of Na^+ transport in A6 cells is mediated via glucocorticoid receptors. *Am. J. Physiol.* **264:**C875–C884
107. Schwabe, J.W.R., Chapman, L., Finch, J.T., Rhodes, D. 1993. The crystal structure of the estrogen receptor DNA-binding domain bound to DNA—how receptors discriminate between their response elements. *Cell* **75:**567–578
108. Smith, D.F., Toft, D.O. 1993. Minireview—steroid receptors and their associated proteins. *Mol. Endocrinol.* **7:**4–11
109. Smith, P.R., Saccomani, G., Joe, E.H., Angelides, K.J., Benos, D.J. 1991. Amiloride-sensitive sodium channel is linked to the cytoskeleton in renal epithelial cells. *Proc. Natl. Acad. Sci. USA* **88:**6971–6975
110. Spooner, P.M., Edelman, I.S. 1975. Further studies on the effect of aldosterone on electrical resistance of toad bladder. *Biochim. Biophys. Acta* **405:**304–314
111. Stanton, B.A. 1986. Regulation by adrenal corticosteroids of sodium and potassium transport in loop of Henle and distal tubule of rat kidney. *J. Clin. Invest.* **78:**1612–1620
112. Steinmetz, P.R. 1993. The reductionist approach to urinary acidification: scalar similarities. *News Physiol. Sci.* **8:**282–286
113. Stewart, P.M., Wallace, A.M., Valentino, R., Burt, D., Shackleton, C.H.L., Edwards, C.R.W. 1987. Mineralocorticoid activity of licorice: 11-Beta-hydroxysteroid dehydrogenase deficiency comes of age. *Lancet* **2:**821–824
114. Stoos, B.A., Náray-Fejes-Tóth, A., Carretero, O.A., Ito, S., Fejes-Tóth, G. 1991. Characterization of a mouse cortical collecting duct cell line. *Kidney Int.* **39:**1168–1175
115. Szerlip, H., Palevsky, P., Cox, M., Blazer-Yost, B. 1991. Relationship of the aldosterone-induced protein, gP70, to the conductive Na^+ channel. *J. Am. Soc. Nephrol.* **2:**1108–1114
116. Tang, M.J., McDonough, A.A. 1992. Low K^+ increases Na^+-K^+-

ATPase alpha-subunit and beta-subunit messenger RNA and protein abundance in cultured renal proximal tubule cells. *Am. J. Physiol.* **263:**C436–C442

117. Truscello, A., Gäggeler, H.P., Rossier, B.C. 1986. Thyroid hormone antagonizes an aldosterone-induced protein: a candidate mediator for the late mineralocorticoid response. *J. Membrane Biol.* **89:**173–183
118. Truscello, A., Geering, K., Gaeggeler, H.P., Rossier, B.C. 1983. Effect of butyrate on histone deacetylation and aldosterone-dependent Na^+ transport in the toad bladder. *J. Biol. Chem.* **258:**3388–3395
119. Truss, M., Bartsch, J., Hache, R.S.G., Beato, M. 1993B. Chromatin structure modulates transcription factor binding to the mouse mammary tumor virus (MMTV) promoter. *J. Steroid Biochem. Mol. Biol.* **47:**1–10
120. Truss, M., Beato, M. 1993. Steroid hormone receptors—interaction with deoxyribonucleic acid and transcription factors. *Endocr. Rev.* **14:**459–479
121. Tsukiyama, T., Becker, P.B., Wu, C. 1994. ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature* **367:**525–532
122. Tully, D.B., Allgood, V.E., Cidlowski, J.A. 1994. Modulation of steroid receptor-mediated gene expression by vitamin B-6. *FASEB J.* **8:**343–349
123. Ueda, K., Okamura, N., Hirai, M., Tanigawara, Y., Saeki, T., Kioka, N., Komano, T., Hori, R. 1992. Human p-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.* **267:**24248–24252
124. Verrey, F. 1990. Regulation of gene expression by aldosterone in tight epithelia. *Semin. Nephrol.* **10:**410–420
125. Verrey, F. 1994. Antidiuretic hormone action in A6 cells: Effect on apical Cl and Na conductances and synergism with aldosterone for NaCl reabsorption. *J. Membrane Biol.* **138:**65–76
126. Verrey, F., Digicaylioglu, M., Bolliger, U. 1993. Polarized membrane movements in A6 kidney cells are regulated by aldosterone and vasopressin/vasotocin. *J. Membrane Biol.* **133:**213–226
127. Verrey, F., Kairouz, P., Schaerer, E., Fuentes, P., Geering, K., Rossier, B.C., Kraehenbuhl, J.P. 1989. Primary sequence of *Xenopus laevis* Na^+-K^+-ATPase and its localization in A6 kidney cells. *Am. J. Physiol.* **256:**F1034–F1043
128. Verrey, F., Kraehenbuhl, J.P., Rossier, B.C. 1989. Aldosterone induces a rapid increase in the rate of Na,K-ATPase gene transcription in cultured kidney cells. *Mol. Endocrinol.* **3:**1369–1376
129. Verrey, F., Schaerer, E., Zoerkler, P., Paccolat, M.-P., Geering, K., Kraehenbuhl, J.P., Rossier, B.C. 1987. Regulation by aldosterone of Na^+,K^+-ATPase mRNAs, protein synthesis, and sodium transport in cultured kidney cells. *J. Cell Biol.* **104:**1231–1237
130. Vilella, S., Guerra, L., Helmle-Kolb, C., Murer, H. 1992. Aldosterone actions on basolateral Na^+/H^+ exchange in Madin-Darby canine kidney cells. *Pfluegers Arch.* **422:**9–15
131. Wade, J.B., Stanton, B.A., Field, M.J., Kashgarian, M., Giebisch, G. 1990. Morphological and physiological response: time course and sodium dependence. *Am. J. Physiol.* **259:**F88–F94
132. Walker, B.R., Campbell, J.C., Williams, B.C., Edwards, C.R.W. 1992. Tissue-specific distribution of the NAD^+-dependent isoform of 11beta-hydroxysteroid dehydrogenase. *Endocrinology* **131:**970–972
133. Wang, Z., Brown, D.D. 1991. A gene expression screen. *Proc. Natl. Acad. Sci. USA* **88:**11505–11509
134. Watlington, C.O., Perkins, F.M., Muson, P.J., Handler, J.S. 1982. Aldosterone and corticosterone binding and effects on Na^+ transport in cultured kidney cells. *Am. J. Physiol.* **242:**F610–F619
135. Wehling, M. 1994. Novel aldosterone receptors—specificity-conferring mechanism at the level of the cell membrane. *Steroids* **59:**160–163
136. Welling, P.A., Caplan, M., Sutters, M., Giebisch, G. 1993. Aldosterone-mediated Na/K-ATPase expression is alpha(1) isoform specific in the renal cortical collecting duct. *J. Biol. Chem.* **268:**23469–23476
137. Wiener, H., Nielsen, J.M., Klaerke, D.A., Jørgensen, P.L. 1993. Aldosterone and thyroid hormone modulation of alpha-1-messenger RNA, beta-1-messenger RNA, and Na,K-pump sites in rabbit distal colon epithelium—Evidence for a novel mechanism of escape from the effect of hyperaldosteronemia. *J. Membrane Biol.* **133:**203–211
138. Wills, N.K., Purcell, R.K., Clausen, C., Millinoff, L.P. 1993. Effects of aldosterone on the impedance properties of cultured renal amphibian epithelia. *J. Membrane Biol.* **133:**17–27
139. Wolffe, A.P. 1994. Transcription—in tune with the histones. *Cell* **77:**13–16
140. Wright, A.P.H., Zilliacus, J., McEwan, I.J., Dahlman-Wright, K., Almlof, T., Carlstedt-Duke, J., Gustafsson, J.A. 1993. Structure and function of the glucocorticoid receptor. *J. Steroid Biochem. Mol. Biol.* **47:**11–19
141. Yamamoto, K., Ikeda, U., Seino, Y., Tsuruya, Y., Oguchi, A., Okada, K., Ishikawa, S.E., Saito, T., Kawakami, K., Hara, Y., Shimada, K. 1993. Regulation of Na,K-adenosine triphosphatase gene expression by sodium ions in cultured neonatal rat cardiocytes. *J. Clin. Invest.* **92:**1889–1895
142. Yoshinaga, S.K., Peterson, C.L., Herskowitz, I., Yamamoto, K.R. 1992. Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* **258:**1598–1604