J. Membrane Biol. 187–197 (1978)

Role of RNA in the Action of Aldosterone on Na^+ Transport

B.C. Rossier

Institut de pharmacologie de l'Université de Lausanne, Switzerland

Received 30 November 1977

Summary. Recent data describing the effects of aldosterone on the induction of messenger RNA (=*m*RNA) and ribosomal RNA (=*r*RNA) are reviewed. In the urinary bladder of the toad, aldosterone induces a few specific polyadenylated *m*RNAs (= poly(A)(+)*m*RNA) during the latent period, i.e., 30 to 60 min after hormone addition. Later, i.e., 90 to 240 min after aldosterone addition, 18S and 28S cytoplasmic *r*RNA subunits are also induced. The effect on poly(A)(+)*m*RNA is mineralocorticoid-specific and correlates well with the aldosterone-dependent Na^+ transport. Actinomycin D which inhibits both poly(A)(+)*m*RNA and nonpolyadenylated *m*RNA (=poly(A)(−)*m*RNA) totally abolishes the response to aldosterone on Na^+ transport. 3′deoxyadenosine (cordycepin), which inhibits poly(A)(+)*m*RNA but not poly(A)(−)*m*RNA, only inhibits 50 to 60% of the physiological response. These differential effects suggest that an intact poly(A)(−)*m*RNA pathway is also an important factor in mediating the action of aldosterone. In contrast, 3′deoxycytidine, which inhibits *r*RNA but not *m*RNA, does not impair the mineralocorticoid response, at least during the first 3 hr of aldosterone action.

A major action of aldosterone is to promote Na^+ reabsorption across a variety of epithelia. This effect appears to be mediated by a cascade of events as summarized in Fig. 1 (*see* recent reviews by Feldman, Funder & Edelman, 1972, and Edelman, 1975). The most widely accepted mode of action of this hormone, the so-called *induction hypothesis,* postulates three major events:

1) *Pretranscriptional steps:* Migration of D-Aldosterone from the blood capillary into the cytoplasm of the target cell; binding of the steroid to a stereospecific cytoplasmic receptor, and transfer of this complex to chromatin acceptor sites.

2) *A transcriptional and/or posttranscriptional step:* Induction of the synthesis of different types of RNA, such as *m*RNA or *r*RNA.

3) *A translational step:* Translation of the induced *m*RNAs into their respective proteins, aldosterone-induced proteins (AIPs), which are in turn responsible for the increased Na^+ transport.

0022-2631/78/S040-0187 $02.20

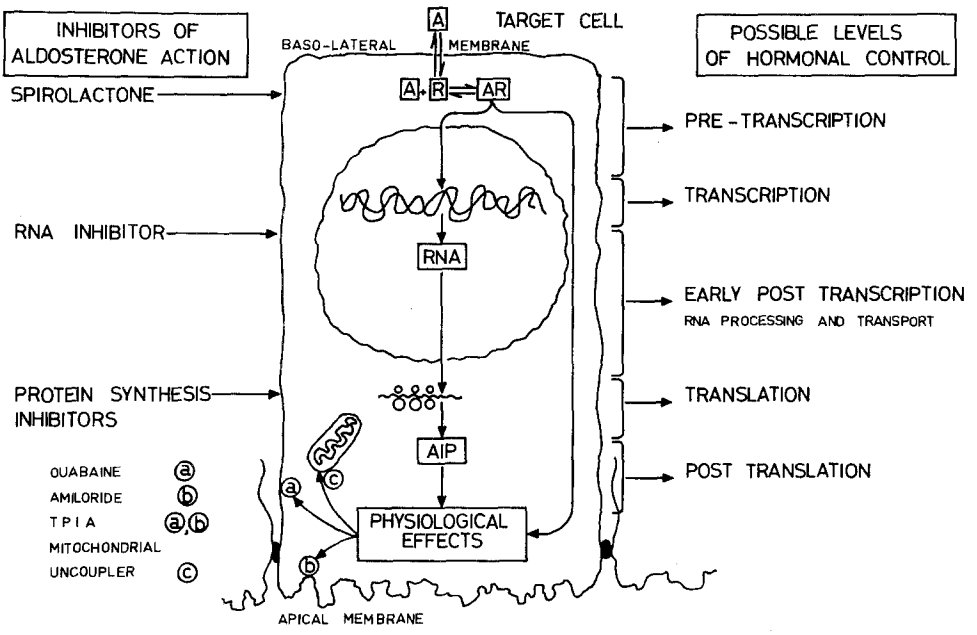

Fig. 1. Models proposed for the mechanism of aldosterone action on Na^+ transport. Aldosterone (*A*) diffuses across the basolateral membrane of a target cell and binds to a stereospecific cytoplasmic receptor (*R*) to generate an active complex (*AR*). *Induction hypothesis:* The active complex enters the nucleus where it binds to chromatin sites leading to the induction of RNA and in turn protein synthesis (aldosterone-induced protein = *AIP*), which ultimately mediates the physiological effect by modifying the permeability of the apical membrane (*b*) or the basolateral membrane (*a*) to sodium or by increasing the supply of energy to the system at the mitochondrial level (*c*). The various levels of hormonal control are indicated on the right. *Noninduction hypothesis:* The possibility of a "pre-transcriptional" effect by-passing the nucleus is indicated: the complex (*AR*) could then have direct effects on (*a*), (*b*) or (*c*). On the left, the major inhibitors of aldosterone action have been listed. *TPIA* stands for 2-methyl-2[p-(1, 2, 3, 4-tetrahydro-l-naphthyl) phenoxy] propionic acid, and inhibitor of lipid synthesis (Lien *et al.* 1975)

The induction of specific *m*RNAs appears to be one of the key factors in the action of aldosterone and of a variety of steroid hormones (King & Mainwaring, 1974). During the last five years or so, considerable evidence has been adduced to support the concept that steroid hormones increase protein synthesis, primarily by the regulation of *m*RNA induction (Rosen & O'Malley, 1975; Schimke, McKnight & Shapiro, 1975), and that they promote the synthesis and the accumulation of *r*RNA (King & Mainwaring, 1974). Within this framework, I wish to review the data that point to a role of aldosterone in the induction of *m*RNA and *r*RNA and try to relate them to the mineralocorticoid action of the hormone.

Action of Aldosterone on Incorporation of Labeled Precursors into Total, Nuclear, and Cytoplasmic RNA

Indirect evidence for the induction hypothesis came first from the use of inhibitors of RNA synthesis, such as Actinomycin D (Edelman, Bogoroch & Porter, 1963; Williamson, 1963; Lahav, Dietz & Edelman, 1973; Lifschitz, Schrier & Edelman, 1973) or 3′deoxyadenosine (cordycepin) (Chu & Edelman, 1972). Both inhibitors effectively block the response of Na^+ transport to aldosterone in the toad bladder and the rat kidney. However, this type of experiment does not indicate whether aldosterone induces the synthesis of specific RNAs *de novo* or whether the synthesis of the macromolecules (especially of short-lived *m*RNAs and/or proteins) is simply necessary ("permissive" effect) to trigger the aldosterone response by another pathway. This indeed represents an entirely different hypothesis for the action of aldosterone, and is indicated in Fig. 1 as the *noninduction hypothesis*.

The possibility that aldosterone induces *de novo* RNA synthesis has been studied in a variety of target cells. A common experimental approach has been to measure the incorporation of labeled precursors (mostly ^{3}H-uridine or ^{3}H-orotic acid) into total RNA or into crude nuclear or cytoplasmic RNA fractions. In many studies, aldosterone increased the incorporation of precursors into RNA, sometimes enhanced the turnover of RNA, in the urinary bladder of the toad (Porter, Bogoroch & Edelman, 1964; Rousseau & Crabbé, 1968; Rousseau & Crabbé, 1972; Hutchinson & Porter, 1972, 1975; Rossier, Wilce & Edelman, 1974; Wilce, Rossier & Edelman, 1976*a*) the rat kidney (Fimognari, Porter & Edelman, 1967; Forte & Landon, 1968; Mishra & Feltham, 1975), the mouse kidney (Suzuki, Ogawa & Inoue, 1976) or the rat intestine (Watts & Wheldrake, 1976).

In the majority of these studies, the magnitude of the aldosterone effect on the incorporation of labeled precursors into total, nuclear, or cytoplasmic RNA was small and ranged from 10 to 30%, depending on the type of precursor and labeling. However, no increase in chemical RNA could be observed in the first 20 hr after aldosterone addition (Hutchinson & Porter, 1972).

In three studies, the effect of aldosterone was either inconsistent or not significant, even on examining RNA fractionated by sucrose density gradients or by polyacrylamide gel electrophoresis (Sharp & Komack, 1971; Vančura, Sharp & Malt, 1971; Rousseau & Crabbé, 1972). These discrepancies are perhaps not so surprising when it is considered that

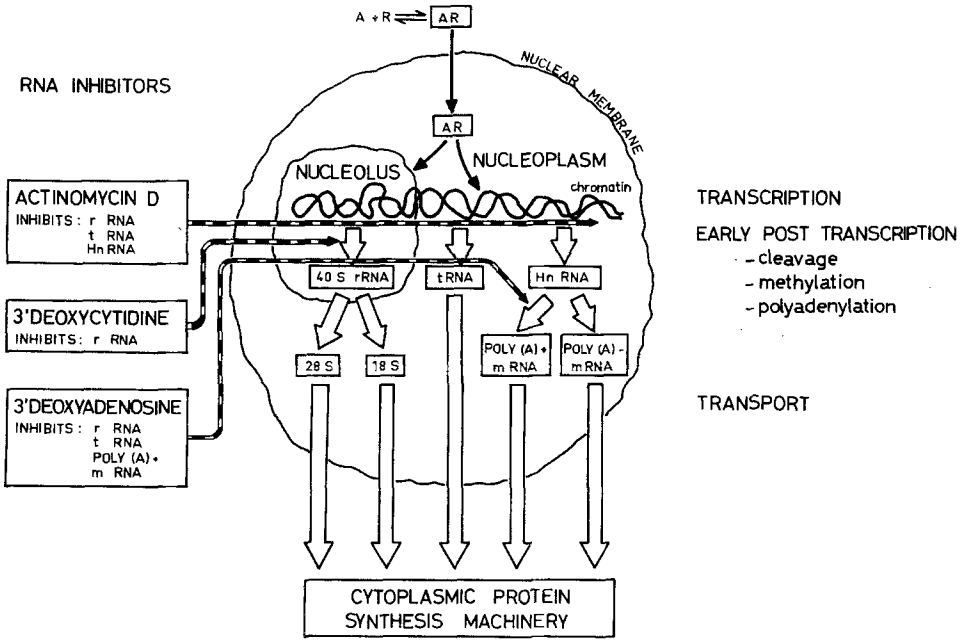

Fig. 2. General model of RNA transcription in an amphibian nucleus. The transcription of the three major classes of RNA (*r*RNA, *t*RNA and *m*RNA) is depicted. On the left, three inhibitors of RNA synthesis are indicated: (i) *Actinomycin D* inhibits the transcription of all classes of RNA (ii) *3'deoxyadenosine* inhibits transcription of *r*RNA and *t*RNA, and the posttranscriptional polyadenylation of *m*RNA. The production of Poly(A) (−)*m*RNA is not inhibited (iii) *3'deoxycytidine* selectively inhibits the *r*RNA pathway. *See text* for explanation

aldosterone might induce the synthesis of only a few classes of *m*RNA, which do not overall represent more than 2 to 5% of the total cell RNA.

To detect such modest effects raises difficult technical and methodological problems. Fig. 2 is a very simplified model of RNA transcription in an eukaryotic cell. On this diagram, the synthesis of the three major RNA classes in an amphibian cell are represented: ribosomal RNA (*r*RNA), transfer RNA (*t*RNA) and messenger RNA (*m*RNA). Ribosomal RNA is synthesized in the nucleolus as a heavy precursor (40S in amphibian), where it is methylated to a high degree and cleaved into nuclear 28S and 18S *r*RNA subunits. Finally, after a lag period of about 90 min, it passes into the cytoplasmic protein synthesis machinery. Transfer RNA is synthesized in the nucleoplasm, methylated,

and rapidly transferred into the cytoplasm. Ribosomal RNA and transfer RNA constitute up to 95% of the cellular (chemical) RNA. Messenger RNA derives apparently from a heavy precursor (presumably the heterogeneous nuclear RNA, i.e., HnRNA) which is rapidly processed by cleavage and minimal but specific methylation at the 5′end of the molecule ("capping"). The 3′ends of some of the molecules are then polyadenylated. This posttranscriptional modification of the *m*RNA molecules allows the definition of two distinct classes of *m*RNA: (i) the poly-(A)(+)*m*RNAs which code for most of the cellular and secretory proteins, and (ii) the poly(A)(−)*m*RNA (nonpolyadenylated molecules) which code for proteins such as histones. Both classes of *m*RNA together represent at most 2 to 4% of the cell RNA. If aldosterone induces only a limited number of specific *m*RNAs, it is easily understood why they cannot be detected without using both adequate labeling and purification techniques.

Action of Aldosterone on Poly (A) (+) mRNA

Recently we reported that aldosterone increased the incorporation of 5-^{3}H-uridine into a class of minimally methylated, rapidly labeled cytoplasmic RNAs ("putative *m*RNA") that sedimented at 9S to 12S on nondenaturing sucrose density gradients (Rossier *et al.*, 1974). This effect was apparent during the latent period (first 60 min) before the hormone exerted its physiological action and was also observed at 120 to 180 min when using a pulse chase labeling technique. A 30 min ^{3}H-uridine pulse and a chase of a thousand fold excess of unlabeled precursor for 90–150 min favored the detection of a transitory effect of aldosterone. In another study (Wilce *et al.*, 1976*a*), we reported that aldosterone increased the incorporation of ^{3}H-uridine and ^{3}H-adenosine (30′ pulse and 150′chase format) into cytoplasmic poly(A)(+)RNA isolated by oligo(dT)-cellulose chromatography. A major effect was observed in a class of poly(A)(+)RNAs that sedimented at 12S on nondenaturing sucrose density gradients, and to a lesser extent at 7S and 18S. The effect of aldosterone was most marked during the first 30 min of hormone action. A large increase in the incorporation of ^{3}H-uridine into poly-(A)(+)RNA extracted from the epithelial cell of the urinary bladder of the toad has also been reported in two preliminary studies, where the effect was particularly pronounced in mitochondria-rich cells (Scott & Sapirstein, 1974; Brown & Scott, 1976).

Table 1. Effects of aldosterone on Na^+ transport and poly(A)(+)RNA at steady state

Condition	Na^+ transport μA/hemibladder		[^{3}H]poly(U) · poly(A) hybrid	
	SCC_0	SCC_{12h}	Nucleus [^{3}H]cpm/μg DNA	Cytoplasm [^{3}H]cpm/μg RNA
Control	224 ±25	234 ±17	46 ±5.4	203 ±5.9
Aldosterone	227 ±29	396 ±29	57 ±5.2	221 ±5.6
Fractional change A/C	1.03±0.06	1.72±0.08	1.240±0.100	1.090±0.014
Paired *t* test	NS	$p<0.001$	$p<0.05$	$p<0.01$

60 bladders, divided into 24 paired pools of 5 hemibladders each were incubated in (HCO_3^-) Ringer solution either in presence of the diluent (control) or in presence of $7 \cdot 10^{-8}$ M aldosterone for 12 hours at 25 °C. Sodium transport was measured by the short-circuit current technique as previously described (Rossier et al., 1974). At the end of the incubation, total nuclear RNA and cytoplasmic RNA were extracted, as indicated previously (Rossier et al., 1974). Thirty μg aliquots (in triplicate) of nucleic acid were hybridized with [^{3}H]poly(U) (specific activity 63 μCi/μMPi) for 30 min at 0 °C, as described by Rosbash and Ford (1974). Hybrids were then digested with ribonuclease and [^{3}H]activity in the poly(A) · poly(U) hybrid was counted. The data are presented as mean ± SEM, $n=10$. In two pools, fractional changes in [^{3}H]poly(U) · poly(A) hybrid deviated by more than 5 and 6 standard deviations respectively and have not been included in the statistics.

The early increase in labeling of the poly(A)(+)RNA induced by aldosterone may result in a net accumulation of these species during the action of the hormone. Hybridization of the unlabeled cytoplasmic or nuclear RNA to a high specific activity ^{3}H-poly(U) probe is an accurate and sensitive method. The basis of the technique (Rosbash & Ford, 1974) is to hybridize the poly(A) of *m*RNA to a tritiated polyuridine oligonucleotide (^{3}H-poly(U)), a complementary sequence, which will form a stable hybrid with poly(A). The poly(A) · Poly(U) hybrid is then separated from the free poly(U) and assayed for radioactivity by scintillation spectrometry. It provides a method to titrate accurately the amount of poly(A) in any biological extract and thus to quantitate the accumulation of a few poly(A)(+)*m*RNAs induced by a steroid. As shown in Table 1, aldosterone increased Na^+ transport by 72% ($P<0.001$) after 12 hr, the amount of cytoplasmic poly(A) by 9% ($P<0.01$) and nuclear poly(A) by 24% ($P<0.05$). The overall increase in cytoplasmic poly(A), although significant, was very small. However, it corresponded to larger increases in poly(A)*m*RNA of certain sizes when the cytoplasmic RNA was first fractionated on a sucrose density gradient and then challenged in each fraction by the ^{3}H-poly(U) probe.

Aldosterone increased the amount of cytoplasmic poly(A) in three distinct RNA classes one sedimenting at 9S (+41%), one at 12S (+10%) and one at 18S (+22%).

Comparable results but with a slightly different hybridization technique have been previously reported (Wilce *et al.*, 1976*a*). Overall, the data presented above are consistent with the inference that aldosterone stimulates poly(A)(+)RNA synthesis and/or rapidly stabilizes their turnover, leading to the accumulation of certain classes of *m*RNA in the cell. The number and the types of protein coded by these induced *m*RNAs are not known at the present time. Many recent reports (Benjamin & Singer, 1974; Scott & Sapirstein, 1975) indicate that aldosterone enhances the incorporation of labeled precursors into various proteins of different sizes. The function and the precise subcellular localization of these induced proteins have not yet been identified. A notable exception is the induction of citrate synthase in rat kidney mitochondria as reported by Law and Edelman (*upublished report*). As citrate synthase appears also to be induced in the toad bladder system (Kirsten, Kirsten & Sharp, 1970), it now becomes possible to explore the link between induced *m*RNAs and induced proteins, providing that monospecific antibodies can be raised against the citrate synthase. This would allow the establishment of a biological assay for the citrate synthase *m*RNA activity.

Role of mRNA in the Response of Na^+ Transport to Aldosterone

The results discussed imply that the induction of *m*RNA by aldosterone mediates its effect on Na^+ transport. However, no direct experimental evidence for such a link is presently available but circumstantial evidence summarized below speaks for such a relationship. The effect of aldosterone on the incorporation of ^{3}H-uridine into cytoplasmic RNA: (i) is observed at a physiological concentration of hormone and during the latent period *before* any significant change in Na^+ transport; (ii) is not induced by 17α-isoaldosterone (Rossier *et al.*, 1974), a stereoisomer which does not bind to the cytoplasmic receptor (Herman, Fimognari & Edelman, 1968); (iii) is not induced by cortisol (Rossier *et al.*, 1974), a glucocorticoid which is effective in the toad; and (iv) is antagonized by spironolactone (SC 9420) (Hutchinson & Porter, 1970; Rossier *et al.*, 1974; Rossier, Wilce & Edelman, 1977*a*), a competitive inhibitor of aldosterone for its effect on Na^+ transport (Porter, 1968) and its cytoplasmic receptor (Marver *et al.*, 1974). Moreover, by varying the molar ratio of agonist to antagonist and observing the change in Na^+ transport

and incorporation of uridine into 12S cytoplasmic RNA, a linear relationship between these two parameters was observed (Rossier *et al.*, 1977*a*).

So far we have only considered the possible role of aldosterone-induced poly(A)(+)*m*RNA. More recently, we have obtained indirect evidence that the poly(A)(−)*m*RNA could also be an important factor in mediating the Na^+ transport response. This inference is based on the differential effect of two well-characterized inhibitors of RNA synthesis: Actinomycin D and 3′deoxyadenosine (cordycepin) (*see* Fig. 2). Actinomycin D (2 μg/ml in the incubation medium) inhibited 70 to 75% of the incorporation of ^{3}H-uridine into poly(A)(+)RNA and totally abolished the response of Na^+ transport to aldosterone. 3′deoxyadenosine (30 μg/ml) produced an equivalent inhibition of ^{3}H-uridine incorporation into poly(A)(+)RNA but significantly inhibited the sodium response only by 50%. A major difference between these inhibitors (*see* Fig. 2) is the fact that Actinomycin D inhibits the synthesis of both poly(A)(+) and poly(A)(−)*m*RNA while 3′deoxyadenosine leaves that of poly(A)(−)*m*RNA intact. It is not yet clear whether the effect of aldosterone on poly(A)(−)*m*RNA is inductive or "permissive".

Action of Aldosterone on rRNA

Different studies provide indirect evidence that aldosterone also promotes the synthesis of *r*RNA. For instance, aldosterone increased RNA polymerase I activity (the nucleolar enzyme for *r*RNA transcription) (Liew, Lin & Gornall, 1972) and the RNA polymerase I/II activity ratio (Chu & Edelman, 1972) in rat kidney nuclei. In the toad bladder, aldosterone-stimulated methylation of nuclear *r*RNA (18S, 28S, and 40S) by [methyl-^{3}H]methionine within 30 min and of 28S cytoplasmic *r*RNA subunits within 90 min of continuous exposure to the precursor and the hormone (Wilce, Rossier & Edelman, 1976*b*). In addition the incorporation of ^{14}C-uridine into cytoplasmic 4S *t*RNA, 18S and 28S *r*RNA was enhanced after 240 min of continuous exposure to both precursor and hormone. Aldosterone had minimal effects on the ^{3}H and ^{14}C labeled acid-soluble pool. Taken together these results suggest that aldosterone augments the synthesis of *r*RNA. The effect is observed very early at the nuclear level but is only detectable after 90 min in the cytoplasm.

Role of rRNA in the Response to Aldosterone

The appearance of newly synthesized *r*RNA subunits in the cytoplasm at the end of the latent period suggests that a coordinate synthesis of

*m*RNA and *r*RNA may be of importance in the onset of the Na^+ response. To assess this possibility, we took advantage of 3′deoxycytidine, an inhibitor of the synthesis of *r*RNA that had minimal effects on that of *m*RNA (Rossier *et al.*, 1977*b*). We observed that 3′deoxycytidine did not inhibit the response of Na^+ transport to aldosterone for at least 3 hr. The initial mineralocorticoid response is thus not dependent on an intact *r*RNA pathway. However, the action of aldosterone on *r*RNA metabolism could play a role in sustaining the response for longer periods, but this hypothesis has not yet been explored.

Conclusion

We have briefly reviewed the evidence for the induction of the synthesis of RNAs by aldosterone and their possible role in the mineralocorticoid action of the hormone. We have deliberately left aside the possibility that aldosterone might also have effects on Na^+ transport which are not mediated by the genetic pathway, for instance on lipid metabolism (Goodman, Allen & Rasmussen, 1971; Goodman, Wong & Rasmussen, 1975; Lien, Goodman & Rasmussen, 1975), as these have recently been reviewed (Rasmussen, Goodman & Max, 1977). However, as the effect of aldosterone on lipid synthesis is also inhibited by RNA synthesis inhibitors such as 3′deoxyadenosine (Lien, Goodman & Rasmussen, 1976), a direct effect has not yet been clearly demonstrated. Moreover, one should not overlook the complexity of the action of aldosterone which apparently can increase, to a great extent independently, the transport of three different ions: Na^+, K^+ and H^+. The cell type and the biochemical pathways which are involved in these various actions are thus far from being clearly delineated and understood.

Supported in part by grants 3.2630.74 and 3.731.076—Fonds National Suisse de la Recherche Scientifique.

References

Benjamin, W.B., Singer, I. 1974. Aldosterone-induced protein in toad urinary bladder. *Science* **186**:269

Brown, J.A., Jr., Scott, W.N. 1976. Aldosterone induces the synthesis of messenger RNA in mucosal cells of the toad's urinary bladder. *Physiologist* **19**:141

Chu, L.L.H., Edelman, I.S. 1972. Cordycepin and α-amanitine: Inhibitors of transcription as probes of aldosterone action. *J. Membrane Biol.* **10**:291

Edelman, I.S. 1975. Mechanism of action of steroid hormones. *J. Steroid Biochem.* **6**:147

Edelman, I.S., Bogoroch, R., Porter, G.A. 1963. On the mechanism of action of aldosterone on Na^+ transport: The role of protein synthesis. *Proc. Nat. Acad. Sci. USA* **50**:1169

Feldman, D., Funder, J.W., Edelman, I.S. 1972. Subcellular mechanisms in the action of adrenal steroids. *Am. J. Med.* **53**: 545

Fimognari, G.M., Porter, G.A., Edelman, I.S. 1967. Induction of RNA and protein synthesis in the action of aldosterone in the rat. *Am. J. Physiol.* **213**:954

Forte, L., Landon, E.J. 1968. Aldosterone-induced RNA synthesis in the adrenalectomized rat kidney. *Biochim. Biophys. Acta* **157**:303

Goodman, D.B.P., Allen, J.E., Rasmussen, H. 1971. Studies on the mechanisms of action of aldosterone: hormone-induced changes in lipid metabolism. *Biochemistry* **10**:3825

Goodman, D.B.P., Wong, M., Rasmussen, H. 1975. Aldosterone-induced membrane phospholipid fatty acid metabolism in the toad urinary bladder. *Biochemistry* **14**:2803

Herman, T.S., Fimognari, G.M., Edelman, I.S. 1968. Studies on renal aldosterone binding proteins. *J. Biol. Chem.* **243**:3849

Hutchinson, J.H., Porter, G.A. 1970. Spirolactone inhibition of aldosterone-induced nuclear RNA synthesis in toad urinary bladder. *Res. Commun. Chem. Path. Pharmacol.* **1**:363

Hutchinson, J.H., Porter, G.A. 1972. The effect of temperature and substrate concentration on the kinetics of 6-^{3}H-uridine incorporation into RNA of toad bladder epithelial cells: The role of aldosterone. *Biochim. Biophys. Acta* **281**:55

Hutchinson, J.H., Porter, G.A. 1975. Accelerated RNA turnover in toad urinary bladder epithelial cells during early aldosterone action. *Physiol. Chem. Phys.* **7**:453

King, R.J.B., Mainwaring, W.I.P. 1974. Steroid-Cell Interactions. Butterworths, London

Kirsten, E., Kirsten, R., Sharp, G.W.G. 1970. Effects of sodium transport stimulating substances on enzyme activities in the toad bladder. *Pfluegers Arch.* **316**:26

Lahav, M., Dietz, T., Edelman, I.S. 1973. The action of aldosterone on Na^+ transport: Further studies with inhibitors of RNA and protein synthesis. *Endocrinology* **92**:1685

Lien, E.L., Goodman, D.B.P., Rasmussen, H. 1975. Effects of an acetyl-coenzyme A carboxylase inhibitor and a sodium-sparing diuretic on aldosterone-stimulated sodium transport lipid synthesis and phospholipid fatty acid composition in the toad urinary bladder. *Biochemistry* **14**:2749

Lien, E.L., Goodman, D.B.P., Rasmussen, H. 1976. Effects of inhibitors of protein and RNA synthesis on aldosterone-stimulated changes in phospholipid fatty acid metabolism in the toad urinary bladder. *Biochim. Biophys. Acta* **421**:210

Liew, C.C., Lin, D.K., Gornall, A.G. 1972. Effects of aldosterone on RNA polymerase in rat heart and kidney nuclei. *Endocrinology* **90**:488

Lifschitz, M.D., Schrier, R.W., Edelman, I.S. 1973. Effect of actinomycin D on aldosterone-mediated changes in electrolyte excretion. *Am. J. Physiol.* **224**:376

Marver, D., Stewart, J., Funder, J.W., Feldman, D., Edelman, I.S. 1974. Renal aldosterone receptors: Studies with ^{3}H-aldosterone and the anti-mineralocorticoid ^{3}H-spirolactone (SC 26304). *Proc. Nat. Acad. Sci. USA* **71**:1431

Mishra, R.K., Feltham, L.A.W. 1975. RNA polymerase stimulation: Effect of aldosterone and other adrenocorticoids on RNA turnover in rat kidney. *Can. J. Biochem.* **53**:70

Porter, G.A. 1968. *In vitro* inhibition of aldosterone-stimulated Na^+ transport by steroidal spirolactones *Mol. Pharmacol.* **4**:224

Porter, G.A., Bogoroch, R., Edelman, I.S. 1964. On the mechanism of action of aldosterone on Na^+ transport: The role of RNA synthesis. *Proc. Nat. Acad. Sci. USA* **52**:1326

Rasmussen, H., Goodman, D.P.B., Max, E. 1977. Steroid-hormone-induced alterations in membrane lipids—the basis for altered ion transport. *In*: Biochemistry of Membrane Transport. G. Semenza and E. Carafoli, editors. P. 470. (*FEBS* Symposium No. 42). Springer-Verlag, Berlin-Heidelberg-New York

Rosbash, M., Ford, P.J. 1974. Polyadenylic acid-containing RNA in *xenopus laevis* oocytes. *J. Mol. Biol.* **85**:87

Rosen, J.F., O'Malley, B.W. 1975. Hormonal regulation of specific gene expression in the chick oviduct. *In:* Biochemical Actions of Hormone. G. Litwack, editor. Vol. 3, p. 271. Academic Press, New York

Rossier, B.C., Gäggeler, H.P., Rossier, M.C. 1978. Effects of 3′deoxyadenosine and Actinomycin D on RNA synthesis in toad bladder: Analysis of response to aldosterone. *J. Membrane Biol.* **41**:149

Rossier, B.C., Wilce, P.A., Edelman, I.S. 1974. Kinetics of RNA labeling in toad bladder epithelium: Effects of aldosterone and related steroids. *Proc. Nat. Acad. Sci. USA* **71**:3101

Rossier, B.C., Wilce, P.A., Edelman, I.S. 1977*a*. Spironolactone antagonism of aldosterone action on Na^+ transport and RNA metabolism in toad bladder epithelium. *J. Membrane Biol.* **32**:177

Rossier, B.C., Wilce, P.A., Inciardi, J.F., Yoshimura, F.K., Edelman, I.S. 1977*b*. Effects of 3′deoxycytidine on rRNA synthesis in toad bladder: Analysis of response to aldosterone. *Am. J. Physiol.* **232**:*C*174

Rousseau, G., Crabbé, J. 1968. Stimulation by aldosterone of a rapidly labelled RNA fraction in toad bladder tissue. *Biochim. Biophys. Acta* **157**:25

Rousseau, G., Crabbé, J. 1972. Effects of aldosterone on RNA and protein synthesis in the toad bladder. *Eur. J. Biochem.* **25**:550

Schimke, R.T., McKnight, G.S., Shapiro, D.J. 1975. Nucleic acid probes and analysis of hormone action in oviduct. *In:* Biochemical Actions of Hormones. G. Litwack, editor. Vol. 3, pp. 246–270. Academic Press, New York

Scott, W.N., Sapirstein, V.S. 1974. Cellular localisation of hormone action in the toad urinary bladder. *J. Clin. Invest.* **53**:73*A*

Scott, W.N., Sapirstein, V.S. 1975. Identification of aldosterone-induced proteins in the toad's urinary bladder. *Proc. Nat. Acad. Sci. USA* **72**:4056

Sharp, G.W.G., Komack, C.L. 1971. The effects of aldosterone on toad bladder measurements of ^{3}H-uridine incorporation into RNA. *Biochim. Biophys. Acta* **247**:66

Suzuki, S., Ogawa, E., Inoue, Y. 1976. Effects of aldosterone, Actinomycin D, puromycin and cycloheximide on RNA synthesis, carbonic anhydrase and ATPase activities of the kidney and on urinary excretion of sodium in adrenalectomized mice. *J. Steroid Biochem.* **7**:429

Vančura, P., Sharp, G.W.G., Malt, R.A. 1971. Kinetics of RNA synthesis in toad bladder epithelium: Action of aldosterone during the latent period. *J. Clin. Invest.* **50**:543

Watts, R.W., Wheldrake, J.F. 1976. Aldosterone-induced changes in RNA synthesis in rat intestine. *J. Steroid. Biochem.* **7**:263

Wilce, P.A., Rossier, B.C., Edelman, I.S. 1976*a*. Actions of aldosterone on polyadenylated ribonucleic acid and Na^+ transport in the toad bladder. *Biochemistry* **15**:4279

Wilce, P.A., Rossier, B.C., Edelman, I.S. 1976*b*. Actions of aldosterone on rRNA and Na^+ transport in the toad bladder. *Biochemistry* **15**:4286

Williamson, H.E. 1963. Mechanisms of the antinatriuretic action of aldosterone. *Biochem. Pharmacol.* **12**:1449