Mineralocorticoid-specificity of aldosterone-induced protein synthesis in giant-toad (*Bufo marinus*) urinary bladders

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We have identified a group of proteins ($M_r \sim 70\,000$ – $80\,000$; $pI \sim 5.8$ – 6.4) in giant-toad (*Bufo marinus*) urinary-bladder epithelial cells whose synthesis appears to be related to aldosterone-stimulated Na^+ transport. To define this relationship further, we examined whether submaximal natriuretic concentrations of aldosterone induced these proteins and whether spironolactone (a specific mineralocorticoid antagonist in renal epithelia) inhibited their synthesis. Short-circuit current was used to measure Na^+ transport and epithelial-cell protein synthesis was detected with high-resolution two-dimensional polyacrylamide-gel electrophoresis and autoradiography. Submaximal natriuretic concentrations of aldosterone ($1.4 \times 10^{-8} M$) induced the same proteins as maximal concentrations of the hormone ($1.4 \times 10^{-7} M$). In contrast, in previous experiments, similar proteins were not induced by subnatriuretic concentrations ($5.0 \times 10^{-8} M$) of cortisol, a glucocorticoid. A spironolactone/aldosterone molar ratio of 2000:1 was required to inhibit aldosterone-stimulated Na^+ transport completely; ratios of 200:1 and 500:1 produced partial inhibition. Concentrations of spironolactone that abolished aldosterone-stimulated Na^+ transport also inhibited aldosterone-induced protein synthesis. We conclude that the synthesis of the proteins we have identified is specifically related to activation of the mineralocorticoid pathway.

Aldosterone-stimulated Na^+ transport in renal epithelia is mediated by genetic de-repression (Edelman, 1968, 1978). The natriuretic effect of the hormone has been examined in a variety of species, but its cellular mechanism of action has been studied most extensively in the toad urinary bladder. In this 'in vitro' model system, aldosterone binds to specific cytosolic receptors and to two classes of nuclear receptors; occupation of the high-affinity (Type I) nuclear receptor has been correlated with the natriuretic activity of the steroid (Farman *et al.*, 1978; Kusch *et al.*, 1978). Aldosterone has also been shown to increase the synthesis of a species of RNA with characteristics of mRNA (Rossier *et al.*, 1974, 1977, 1978; Rossier, 1978). These data, and the results of studies employing inhibitors of mRNA and protein synthesis (Sharp & Leaf, 1966), strongly suggest that new protein synthesis is a prerequisite for aldosterone-stimulated Na^+ transport. However,

the identification and characterization of specific aldosterone-induced proteins has proven difficult, and although several such proteins have been described (Benjamin & Singer, 1974; Scott & Sapirstein, 1975; Scott *et al.*, 1978, 1979; Law & Edelman, 1978*a,b*; Geheb *et al.*, 1981*a*; Reich *et al.*, 1981), which of these has a unique role in determining the magnitude and time-course of aldosterone-stimulated Na^+ transport is unknown.

One problem has been the lack of a model system in which aldosterone-induced protein synthesis can be reliably detected and related to Na^+ transport. However, by using two-dimensional polyacrylamide-gel electrophoresis and autoradiography of [^{35}S]methionine-labelled proteins we have recently detected putative aldosterone-induced proteins in subcellular fractions of toad urinary-bladder epithelial cells. In this preparation, aldosterone at $10^{-7} M$ increases the synthesis of a characteristic group of four to six membrane-associated proteins ($pI \sim 5.8$ – 6.4 ; $M_r \sim 70\,000$ – $80\,000$) and a single cytosolic protein ($pI \sim 6.0$; $M_r \sim 70\,000$) (Geheb *et al.*, 1981*b*). The induction of these proteins seems to be necessary for expression of the hormone's

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natriferic effect. For example, actinomycin D (at a concentration that blocks both aldosterone-stimulated Na^+ transport and new mRNA production) abolishes their synthesis (Geheb *et al.*, 1981b). In addition, their induction is not a non-specific consequence of enhanced epithelial Na^+ transport. For example, vasopressin-stimulated Na^+ transport is not associated with the synthesis of similar proteins, and the proteins are induced even when Na^+ transport is eliminated by preincubation with amiloride (Geheb *et al.*, 1981b).

In the present paper we consider whether the synthesis of these proteins is specifically related to activation of the mineralocorticoid pathway or whether their induction is merely a generalized steroid effect. We have examined whether sub-maximal natriferic concentrations of aldosterone induce similar proteins and whether spironolactone (a specific mineralocorticoid antagonist in renal epithelia) (Corval *et al.*, 1981) inhibits their synthesis. Our results indicate that the synthesis of the proteins we have identified is related to activation of the mineralocorticoid pathway.

Materials and methods

Female giant toads (*Bufo marinus*) from the Dominican Republic (National Reagents, Bridgeport, CT, U.S.A.) were maintained in the unfed state in distilled water for at least 48 h before use. The animals were killed by double pithing and the bladders were surgically removed. In those experiments in which only electrophysiological studies were performed, bladders from a single toad were mounted in modified Ussing chambers to yield four matched quarter-bladders. In the combined electrophysiological and biochemical experiments, the bladders were mounted as sacs (mucosa inside) on glass tubes to yield two matched hemibladders (Geheb *et al.*, 1981b). The modified Ringer's solution used in the isolated electrophysiological studies, and the incubation medium used in the combined electrophysiological and biochemical studies, were identical with those previously employed (Guzzo *et al.*, 1978; Geheb *et al.*, 1981b). Potential difference was measured with calomel reference electrodes and short-circuit current was used to measure Na^+ transport (Ussing & Zerahn, 1951). Stock solutions of aldosterone (Sigma) were prepared in methanol (2.8×10^{-3} M) and stored at 4°C. Stock solutions of spironolactone (SC 9420; Sigma) were prepared in methanol (5.6×10^{-3} M) or dimethyl sulfoxide (1.4×10^{-2} M) and stored at 4°C.

In the isolated electrophysiological studies, after a period of stabilization (1–3 h), aldosterone, spironolactone and the appropriate carriers were added to individual quarter-bladders as needed; protocol

details are provided in the legends to Fig. 1 and Table 1. Short-circuit current was then continuously monitored for the remainder of the experiment.

In the combined electrophysiological and biochemical experiments, protocols similar to those employed previously (Geheb *et al.*, 1981b) were used to evaluate the effects of aldosterone and spironolactone on new protein synthesis; details are provided in the legends to Figs. 3 and 4. In each protocol, [^3S]methionine (specific radioactivity 800–1400 Ci/mmol; Amersham International) was added to the serosal medium of both hemibladders (final concentration 100–125 $\mu\text{Ci/ml}$) 6 h after the addition of aldosterone, aldosterone and spironolactone, or carrier(s), and the incubation was continued for an additional 4 h. Potential difference was monitored continuously and short-circuit current was measured at approx. 2 h intervals.

At the end of the isotope incubation period, each bladder was rinsed in cold (4°C) incubation medium and the epithelial cells were collected by scraping and homogenized in 2 ml of a solution containing 220 mM-sucrose, 2 mM-EDTA and 5 mM-Tris (pH 7.5) with a tight-fitting Dounce homogenizer (40 strokes). The homogenate was centrifuged (800 g, 5 min) to remove cellular debris and the resulting supernatant was centrifuged (130 000 g, 1 h) to yield membrane-rich (pellet) and cytosolic (supernatant) fractions. The cytosol was dialysed at 4°C against 5 mM-Tris/HCl, pH 7.5, for 16–20 h (using 2 kDa-cut-off tubing) and then freeze-dried.

New protein synthesis was identified by two-dimensional polyacrylamide-gel electrophoresis and autoradiography as previously described (Geheb *et al.*, 1981b; O'Farrell, 1975). For each pair of fractions (control and experimental) in a given experiment, samples of radioactive material with equal numbers of counts were subjected to electrophoresis. Protein contents were measured by the method of Lowry *et al.* (1951). Isoelectric points and molecular weights were estimated as described previously (Geheb *et al.*, 1981b).

Results

Effects of aldosterone and spironolactone on Na^+ transport

A concentration of spironolactone that completely inhibited aldosterone-stimulated Na^+ transport but had no effect on basal Na^+ transport was sought in an extensive series of preliminary electrophysiological experiments (Table 1). An antagonist (spironolactone; 2.8×10^{-5} M)/agonist (aldosterone; 1.4×10^{-7} M) molar ratio of 200:1 was initially employed, but under these conditions there was incomplete inhibition of aldosterone-stimulated Na^+ transport (Table 1, Protocol A). In order to achieve

Table 1. *Effects of spironolactone on basal and aldosterone-induced Na⁺ transport*

In all protocols, hemibladders were mounted in modified Ussing chambers to yield four electrically matched quarter-bladders (QB). After a period of stabilization (1–3 h), aldosterone (Aldo), spironolactone (Spiro) and carriers (methanol, dimethyl sulphoxide) were added to the appropriate quarter-bladders (at 0 h). Short-circuit current (SCC) was then continuously monitored for 10 h. Short-circuit-current values (means $\pm$ S.E.M.) are provided at 0 h and at 1, 6, and 10 h thereafter. Statistical comparisons were performed by using the Student *t* test for paired values. Superscripts (*, †, §) indicate $P < 0.05$ or better (* = QB 1 versus QB 2; † = QB 3 versus QB 4; § = QB 4 versus QB 2).

Protocol	Spiro/Aldo	QB	Additions	Time (h)	...	SCC ($\mu\text{A} \cdot \text{cm}^{-2}$)			
						0	1	6	10
A (<i>n</i> = 11)	200:1	1	Aldo ($1.4 \times 10^{-7}\text{M}$)			38 ± 7	39 ± 8	$34 \pm 7^*$	$34 \pm 6^*$
		2	Control			38 ± 7	40 ± 7	20 ± 6	10 ± 3
		3	Aldo + Spiro			38 ± 6	31 ± 5	26 ± 6	$22 \pm 5^\dagger$
		4	Spiro ($2.8 \times 10^{-5}\text{M}$)			38 ± 8	$33 \pm 7^\S$	22 ± 5	14 ± 3
B (<i>n</i> = 5)	500:1	1	Aldo ($1.4 \times 10^{-7}\text{M}$)			21 ± 3	22 ± 4	$27 \pm 5^*$	$25 \pm 5^*$
		2	Control			20 ± 5	23 ± 5	9 ± 3	4 ± 1
		3	Aldo + Spiro			21 ± 3	22 ± 2	21 ± 5	$17 \pm 3^\dagger$
		4	Spiro ($7.0 \times 10^{-5}\text{M}$)			19 ± 5	23 ± 6	12 ± 4	6 ± 1
C (<i>n</i> = 6)	2000:1	1	Aldo ($1.4 \times 10^{-7}\text{M}$)			40 ± 10	43 ± 9	$52 \pm 10^*$	$52 \pm 9^*$
		2	Control			35 ± 7	33 ± 5	29 ± 6	18 ± 6
		3	Aldo + Spiro			40 ± 8	20 ± 3	19 ± 3	15 ± 3
		4	Spiro ($2.8 \times 10^{-4}\text{M}$)			39 ± 14	$20 \pm 5^\S$	20 ± 6	13 ± 3
D (<i>n</i> = 8)	500:1	1	Aldo ($5.0 \times 10^{-8}\text{M}$)			33 ± 3	35 ± 3	$34 \pm 4^*$	$33 \pm 3^*$
		2	Control			33 ± 5	36 ± 4	18 ± 3	9 ± 2
		3	Aldo + Spiro			33 ± 7	31 ± 6	26 ± 4	$18 \pm 3^\dagger$
		4	Spiro ($2.8 \times 10^{-5}\text{M}$)			33 ± 7	30 ± 5	25 ± 4	$13 \pm 3^\S$
E (<i>n</i> = 7)	2000:1	1	Aldo ($1.4 \times 10^{-8}\text{M}$)			35 ± 5	34 ± 4	$42 \pm 4^*$	$36 \pm 3^*$
		2	Control			37 ± 5	40 ± 4	20 ± 3	8 ± 1
		3	Aldo + Spiro			34 ± 5	34 ± 5	23 ± 3	9 ± 1
		4	Spiro ($2.8 \times 10^{-5}\text{M}$)			33 ± 5	31 ± 4	23 ± 3	$10 \pm 1^\S$

complete inhibition of aldosterone-stimulated Na⁺ transport the concentration of spironolactone was progressively increased while that of aldosterone was kept the same ($1.4 \times 10^{-7}\text{M}$). At an antagonist/agonist molar ratio of 500:1, the inhibition of aldosterone-stimulated Na⁺ transport remained incomplete (Table 1, Protocol B), but at a ratio of 2000:1, aldosterone-stimulated Na⁺ transport was abolished (Table 1, Protocol C).

Complete inhibition of aldosterone-stimulated Na⁺ transport was also achieved by decreasing the concentration of aldosterone while keeping that of spironolactone constant ($2.8 \times 10^{-5}\text{M}$). At an antagonist/agonist molar ratio of 500:1, incomplete inhibition was again observed (Table 1, Protocol D), whereas at a ratio of 2000:1, inhibition was complete (Table 1, Protocol E; Fig. 1). The concentration of aldosterone employed in Protocol E ($1.4 \times 10^{-8}\text{M}$), both in our hands (Fig. 2) and in the experience of others (Sharp & Leaf, 1966; Farman *et al.*, 1978), is a submaximal natriuretic concentration of the hormone.

The high concentration of spironolactone employed in Protocol C ($2.8 \times 10^{-4}\text{M}$) had an early and transient effect on basal Na⁺ transport (Table 1).

Inhibition was evident within 30–60 min, but, after 6 h, Na⁺ transport in control and spironolactone-treated quarter-bladders was similar and the drug had no effects on Na⁺ transport over the subsequent 4 h. Concentrations of spironolactone less than $2.8 \times 10^{-4}\text{M}$ generally has little or no effect on basal Na⁺ transport (Protocols B, D and E). The one exception was Protocol A, in which spironolactone at $2.8 \times 10^{-5}\text{M}$ had a small (and transient) inhibitory effect at 1 h.

Effects of aldosterone and spironolactone on new protein synthesis

To evaluate the effects of spironolactone on aldosterone-induced protein synthesis, an antagonist/agonist molar ratio that completely inhibited aldosterone-stimulated Na⁺ transport (2000:1, Table 1) was employed. Two separate series of experiments were performed: one used submaximal ($1.4 \times 10^{-8}\text{M}$), and one used maximal ($1.4 \times 10^{-7}\text{M}$), natriuretic concentrations of the hormone. At a concentration of $1.4 \times 10^{-7}\text{M}$, aldosterone induced the same characteristic 'cluster' of four to six proteins ($pI \sim 5.8$ – 6.4 ; $M_r \sim 70\,000$ – $80\,000$) in the

Fig. 1. Effects of spironolactone on basal and aldosterone-induced Na⁺ transport (Protocol E)

Both hemibladders (from a single toad) were mounted in modified Ussing chambers to provide four electrically matched quarter-bladders. Aldosterone (●), aldosterone and spironolactone (○), spironolactone (□), or carrier (control; ■) were added to the serosal medium of the appropriate quarter-bladder at 0h, and short-circuit current was continuously monitored for 10h. The concentrations of aldosterone and spironolactone were 1.4×10^{-8} M and 2.8×10^{-5} M respectively (antagonist/agonist molar ratio 2000:1). The ordinate represents short-circuit current (SCC) in $\mu\text{A} \cdot \text{cm}^{-2}$ (mean $\pm$ S.E.M.; $n = 7$) and the abscissa represents time. A large natriergic response (relative to control) is evident in the aldosterone-treated quarter-bladder ($P < 0.05$ or better at 4, 6, 8 and 10h). In contrast, the short-circuit current of the quarter-bladders treated either with aldosterone and spironolactone or with spironolactone alone was not different from that of the control at any time period. Consequently, at these concentrations, spironolactone completely and selectively inhibited aldosterone-induced Na⁺ transport.

130000g membrane-rich pellet as had been described previously in 15000g and 120000g pellets (Geheb *et al.*, 1981b) (e.g., Fig. 3a). [A single membrane-rich fraction was used in the present experiments since we have shown that the two fractions employed previously were markedly cross-contaminated (Geheb *et al.*, 1981b).] In addition, as in our previous experiments, this concentration of aldosterone induced the synthesis of a single cytosolic

Fig. 2. Effects of aldosterone on Na⁺ transport (dose-response relationship)

Hemibladders were mounted in modified Ussing chambers to provide two quarter-bladders. Different concentrations of aldosterone (shown on the abscissa) were added to one quarter-bladder at zero time and the other quarter-bladder served as a control. Short-circuit current was then monitored continuously for 10h. The short-circuit current (SCC) 10h after the addition of aldosterone (○) or carrier (■) is shown on the ordinate (mean $\pm$ S.E.M., $n = 8$ for each concentration of hormone). Maximal, and approximately half-maximal, natriergic responses to aldosterone are evident at $\sim 10^{-7}$ M and $\sim 10^{-8}$ M respectively.

protein (pI ~ 6.0 ; $M_r \sim 70000$) (e.g., Fig. 4a). In four separate experiments, a concentration of spironolactone that completely inhibited aldosterone-stimulated Na⁺ transport (2.8×10^{-5} M) eliminated aldosterone-induced protein synthesis in both the membrane-rich and the cytosolic fractions (Figs. 3c, 3d, 4c and 4d).

Similar results were obtained when the concentration of aldosterone was decreased 10-fold (to 1.4×10^{-8} M). The characteristic membrane-associated proteins were evident in five out of seven experiments. However, their autoradiographic grain density appeared to be less than that seen when higher concentrations of aldosterone were employed. In addition, at this concentration of aldosterone, the cytosolic protein was inconsistently induced, being clearly evident in only about half of the experiments. Spironolactone (2.8×10^{-5} M) abolished the synthesis of the membrane-associated proteins in five out of six separate experiments in which 1.4×10^{-8} M aldosterone was employed. Because of the inconsistent induction of the cytosolic protein with this concentration of the hormone, the effects of spironolactone on its synthesis could not be examined in this series of experiments.

Fig. 3. *Effects of spironolactone on aldosterone-induced protein synthesis in membrane-rich fractions*

Autoradiographs of newly synthesized proteins in membrane-rich fractions (130 000 g pellet) of epithelial cells were prepared as described in the Materials and methods section. Equal amounts of radioactivity were placed on all four gels (1.5×10^6 c.p.m.) and the exposure time was the same (7 days) in all cases. (a) and (b) represent paired hemibladders incubated with (a) or without (b) aldosterone (1.4×10^{-7} M). (c) and (d) represent paired hemibladders incubated either with aldosterone (1.4×10^{-7} M) alone (c) or with both aldosterone (1.4×10^{-7} M) and spironolactone (2.8×10^{-4} M) (d). The characteristic membrane-associated proteins are present in the hemibladders treated with aldosterone alone (a and c), but are absent from the control hemibladder (b) and the hemibladder treated with both aldosterone and spironolactone (d).

Discussion

The relationship of the proteins we have identified to aldosterone-induced Na^+ transport has not been completely defined. Since our previous studies (Geheb *et al.*, 1981b) were performed with maximal natriferic concentrations of the hormone (1.4×10^{-7} M), their synthesis could have been due to an undifferentiated steroid effect rather than being the result of activation of the mineralocorticoid-specific pathway. Even though subnatriferic concentrations of cortisol (5×10^{-8} M) did not induce similar proteins (Geheb *et al.*, 1981b), it is possible that the use of a lower concentration of any steroid (including aldosterone) would have yielded negative results. The putative relationship between the induction of these proteins and Na^+ transport would be greatly strengthened if a specific mineralocorticoid antagonist inhibited their synthesis. It would be further strengthened if submaximal natriferic concentrations of aldosterone were shown to induce similar proteins. The present studies were performed with these two goals in mind.

The spiro lactones are specific inhibitors of aldosterone-induced Na^+ transport in renal epithelia (Corval *et al.*, 1981). They competitively inhibit aldosterone-stimulated Na^+ transport in anuran epithelia but have no effect on vasopressin-stimulated Na^+ transport (Porter, 1968; Rossier & Claire, 1978). They inhibit the binding of aldosterone to its cytosolic and nuclear receptors in the rat kidney (Fanestil, 1968; Herman *et al.*, 1968; Marver *et al.*, 1974). In toad urinary bladders, spironolactone (SC 9420) and prorenone displace [^3H]aldosterone from its cytosolic receptor, and such displacement has been correlated with the effects of these two spiro lactones on aldosterone-stimulated Na^+ transport (Rossier *et al.*, 1983). Spironolactone (SC 9420) has also been shown to inhibit aldosterone-induced mRNA synthesis in toad urinary bladders, and the fractional changes in mRNA synthesis have been correlated with the fractional changes in aldosterone-stimulated Na^+ transport (Rossier *et al.*, 1977).

Two groups of investigators have evaluated the effects of spiro lactones on aldosterone-induced

Fig. 4. *Effects of spironolactone on aldosterone-induced protein synthesis in cytosolic fractions*

Autoradiographs of newly-synthesized proteins in cytosolic fractions (130000g supernatant) of epithelial cells were prepared as described in the Materials and methods section. Approximately equal amounts of radioactivity were placed on all four gels (*a*, *b*, 1.5×10^6 c.p.m.; *c*, *d*, 1.1×10^6 c.p.m.). The exposure time of the autoradiographs in (*a*) and (*b*) was 7 days, whereas that of the autoradiographs in (*c*) and (*d*) was 11 days. (*a*) and (*b*) represent paired hemibladders incubated with (*a*) or without (*b*) aldosterone (1.4×10^{-7} M). (*c*) and (*d*) represent paired hemibladders incubated either with aldosterone (1.4×10^{-7} M) alone (*c*) or with both aldosterone (1.4×10^{-7} M) and spironolactone (2.8×10^{-4} M) (*d*). The cytosolic protein is present in the hemibladders treated with aldosterone alone (I; *a* and *c*), but is absent in the control hemibladder (*b*) and in the hemibladder treated with both aldosterone and spironolactone (*d*).

protein synthesis in renal epithelia. Spironolactone (SC 9420) has been shown to inhibit the synthesis of a ~12000 Da protein in toad urinary bladders (Benjamin & Singer, 1974), and another spironolactone (SC 26304) has been shown to inhibit the synthesis of a ~31000 Da aldosterone-induced protein in rat renal medulla (Law & Edelman, 1978a). The spironolactone-sensitivity of these proteins has been used as evidence of their mineralocorticoid-specificity. The particular spironolactone employed in the present studies was spironolactone (SC 9420); this congener has been shown to be a pure mineralocorticoid antagonist (Sakayue & Feldman, 1976).

The preliminary electrophysiological experiments were designed to determine the conditions under which aldosterone-stimulated Na^+ transport was completely inhibited by spironolactone. An antagonist/agonist molar ratio of 2000:1 was necessary before complete inhibition was achieved (Table 1). Concentrations of spironolactone of less than

2.8×10^{-4} M had little or no effect on basal Na^+ transport (Table 1; Fig. 1). Although high concentrations of spironolactone (2.8×10^{-4} M) transiently inhibited basal Na^+ transport, they had no effect on basal Na^+ transport during the period (6–10 h) that new protein synthesis was evaluated. The ability of high concentrations of spironolactone to inhibit transiently basal Na^+ transport has been demonstrated to be mineralocorticoid-specific (Rossier *et al.*, 1983) and has been ascribed to blockade of the natriuretic effect of endogenously released mineralocorticoids (Porter, 1968).

At an antagonist/agonist molar ratio of 2000:1, spironolactone inhibited the induction of the membrane-associated proteins regardless of whether high (Figs. 3 and 4) or low concentrations of aldosterone (1.4×10^{-7} M and 1.4×10^{-8} M respectively) were employed. The cytosolic protein was also inhibited by spironolactone, but this effect could only be adequately evaluated by using the high concentration of the hormone (see the Results section).

Thus, at the same time as aldosterone-stimulated Na^+ transport was selectively and completely inhibited, spironolactone also abolished aldosterone-induced protein synthesis. These results suggest that the synthesis of these proteins is secondary to activation of a mineralocorticoid-specific pathway.

This conclusion is further supported by the fact that concentrations of aldosterone ($1.4 \times 10^{-8} \text{ M}$) that are submaximally natriferric (Fig. 2) induce identical proteins. We have previously reported that concentrations of cortisol that are just subnatriferric ($5.0 \times 10^{-8} \text{ M}$) do not induce proteins similar to those induced by aldosterone (Geheb *et al.*, 1981b). The fact that low concentrations of aldosterone induce these proteins, whereas higher concentrations of a glucocorticoid (cortisol) do not, also supports the mineralocorticoid-specificity of aldosterone-induced protein synthesis.

Although it could not be accurately quantified, the autoradiographic grain density of the proteins induced by the higher concentration of aldosterone appeared to be greater than that of the proteins induced by the lower concentration of the hormone. This suggests the existence of a dose-response relationship between hormone concentration and protein synthesis. The definitive demonstration of such a relationship would strongly support an integral role for these proteins in aldosterone-induced Na^+ transport. Such experiments, however, must await the development of a quantitative assay of aldosterone-induced protein synthesis.

In summary, we have shown that a characteristic group of proteins is induced in toad urinary-bladder epithelial cells in association with aldosterone-induced Na^+ transport. These proteins are synthesized whether maximal or submaximal concentrations of the hormone are employed. Spironolactone, a specific mineralocorticoid antagonist, inhibits both aldosterone-induced Na^+ transport and the synthesis of these proteins. Concentrations of cortisol (a glucocorticoid) 3–4-fold greater than the submaximal concentration of aldosterone employed do not induce similar proteins. These data strengthen the conclusion that the induction of these proteins is not an undifferentiated steroid effect; rather, their synthesis is specifically related to the mineralocorticoid activity of aldosterone.

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