Steroid-induced protein synthesis in giant-toad (*Bufo marinus*) urinary bladders

Correlation with natriferic activity

Michael GEHEB, Robert ALVIS, Alyson OWEN, Eileen HERCKER and Malcolm COX*
Renal-Electrolyte Section, Medical Service, Philadelphia V.A. Medical Center, and Department of Medicine,
University of Pennsylvania School of Medicine, Philadelphia, PA 19104, U.S.A.

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We have identified a group of proteins ($M_r \sim 70000$ – 80000 ; $pI \sim 5.5$ – 6.0) in giant-toad (*Bufo marinus*) urinary bladders whose synthesis appears to be related to aldosterone-stimulated Na^+ transport. Spironolactone, a specific mineralocorticoid antagonist in renal epithelia, inhibits the synthesis of these proteins as well as the natriferic effect of the hormone. Since a variety of other steroids (some of which are traditionally considered to be glucocorticoids) also stimulate Na^+ transport in toad urinary bladders, we examined whether their natriferic activity was expressed in a fashion similar to that of aldosterone. 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. At a concentration of $\sim 100\text{ nM}$, dexamethasone, corticosterone and aldosterone were equinatriferic. Dexamethasone and aldosterone had identical dose–response curves, maximal and half-maximal activity being evident at concentrations of $\sim 100\text{ nM}$ and 10 nM respectively. In contrast, at a concentration of $\sim 10\text{ nM}$, corticosterone had no effect on Na^+ transport. The natriferic activities of these three steroids correlate with their known affinities for the putative mineralocorticoid receptor in toad urinary bladders. Natriferic concentrations of dexamethasone and corticosterone (140 nM) induced the synthesis of proteins with characteristics identical with those induced by aldosterone. Spironolactone, at an antagonist/agonist ratio of 2000:1, inhibited steroid-induced Na^+ transport and the synthesis of these proteins. Thus it appears that all natriferic steroids share a common mechanism of action in toad urinary bladders. Natriferic activity can be correlated not only with relative steroid-receptor affinity but also with the induction of a specific group of epithelial-cell proteins.

Aldosterone-stimulated Na^+ transport in renal epithelia is mediated through genetic de-repression (Edelman, 1968, 1978). In toad urinary bladders, kinetic analysis has demonstrated the presence of both cytosolic and nuclear receptors for aldosterone; occupancy of a high-affinity, low-capacity (Type I) nuclear receptor has been correlated with the ability of aldosterone (and related naturally occurring and synthetic steroids) to stimulate Na^+ transport (Farman *et al.*, 1978; Kusch *et al.*, 1978). Aldosterone has also been shown to increase the

synthesis of mRNA in toad urinary bladders, the induction of which correlates with the hormone's natriferic effect (Rossier *et al.*, 1974, 1977, 1978; Rossier, 1978).

The identification of specific aldosterone-induced proteins has proved more difficult (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). However, by using two-dimensional polyacrylamide-gel electrophoresis and autoradiography of [^{35}S]-methionine-labelled proteins (O'Farrell, 1975), we have detected such proteins in subcellular fractions of toad urinary-bladder epithelial cells. Both maximal ($\sim 100\text{ nM}$) and half-maximal ($\sim 10\text{ nM}$) natriferic concentrations of aldosterone induce a

Abbreviation used: SCC, short-circuit current.

* To whom correspondence and reprint requests should be sent, at the following address: V. A. Medical Center, Room A312R, University and Woodland Avenues, Philadelphia, PA 19104, U.S.A.

characteristic group of four to six membrane-associated proteins ($pI \sim 5.5-6.0$; $M_r \sim 70000-80000$) and a single cytosolic protein ($pI \sim 5.7$; $M_r \sim 70000$) whose synthesis may be necessary for expression of the hormone's natriuretic effect (Geheb *et al.*, 1981b, 1983). The induction of these proteins appears to be related to activation of a mineralocorticoid-specific pathway, since spironolactone, a mineralocorticoid antagonist in renal epithelia (Corval *et al.*, 1981), blocks their synthesis (Geheb *et al.*, 1983) and subnatriuretic concentrations (50 nM) of cortisol, a glucocorticoid, do not induce similar proteins (Geheb *et al.*, 1981b).

The purpose of the present studies was to explore the effects of other steroids (specifically, dexamethasone and corticosterone) on new protein synthesis in toad urinary bladders and to relate these findings to the known affinities of these compounds for the Type I nuclear receptor. Dexamethasone is a potent synthetic glucocorticoid (Haynes & Murad, 1980) and corticosterone is a major endogenous circulating adrenal corticosteroid in the toad (Vinson *et al.*, 1979). Since both steroids stimulate Na^+ transport in toad urinary bladders (Edelman, 1968; Handler *et al.*, 1969), the question arises as to whether their natriuretic activity is related to activation of the mineralocorticoid pathway or to some other, as yet undefined, mechanism. Our results indicate that both dexamethasone and corticosterone stimulate Na^+ transport in toad urinary bladders in a manner indistinguishable from that of aldosterone.

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 electrophysiological studies were performed using electrically matched quarter-bladders (from a single toad) mounted in modified Ussing chambers (Guzzo *et al.*, 1978). In the combined electrophysiological and biochemical experiments, hemi-bladders (from a single toad) were mounted as sacs (mucosa-inside) to yield paired hemi-bladders (Geheb *et al.*, 1981b). Short-circuit current (SCC) was used to measure Na^+ transport, and potential difference was measured with calomel reference electrodes. Stock solutions of aldosterone, dexamethasone and corticosterone were prepared in methanol (2.8 mM) and stored at 4°C. Stock solutions of spironolactone (SC 9420; Sigma) were prepared in dimethyl sulphoxide (14 mM) and stored at 4°C.

In the electrophysiological studies, after a period of stabilization (1-3 h), the test steroids (aldosterone, dexamethasone or corticosterone), with or

without spironolactone and/or the appropriate carriers, were added to individual quarter-bladders as needed. For the dose-response experiments, the same concentrations (from 1.4 nM to 1.4 μ M) of the three test steroids were added to individual quarter-bladders, and the fourth quarter-bladder served as a control. The bladders were then continuously short-circuited for 10 h (see Table 1 below for details). In the experiments evaluating the effects of spironolactone on steroid-induced Na^+ transport, steroid, steroid plus spironolactone, spironolactone alone, or appropriate carriers, were added to individual quarter-bladders. The bladders were then continuously short-circuited for 10 h (see Table 2 below for details).

In the biochemical experiments, similar protocols to those employed previously (Geheb *et al.*, 1981b, 1983) were used to evaluate the effects of the test steroids (aldosterone, dexamethasone, corticosterone) on new protein synthesis. In each protocol, [^{35}S]methionine (specific radioactivity 800-1400 Ci/mmol; Amersham International) was added to the serosal medium of both hemibladders (final concn. 100-125 μ Ci/ml) 6 h after the addition of test steroid or carrier, and the incubation was continued for an additional 4 h. Potential difference was monitored continuously and SCC was measured at approx. 2 h intervals. The effects of spironolactone on steroid-induced protein synthesis were examined in a similar fashion.

Although it might be expected that prolonged incubation *in vitro* (about 12 h) would have deleterious effects on tissue viability, this appears not to be the case with anuran (tail-less-amphibian) epithelia such as the toad urinary bladder. Indeed, bladders have been maintained *in vitro* for 24 h or more without apparent harmful effects (Porter & Edelman, 1964; Spooner & Edelman, 1975, 1976; Siegel & Civan, 1976; Cox *et al.*, 1979; Crabbe, 1980).

At the end of the period of incubation with [^{35}S]methionine, the epithelial cells were collected by scraping with a glass microscope slide, homogenized, and membrane-rich and cytosolic fractions were prepared by ultracentrifugation. Homogenization was performed 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 (800g, 5 min) to remove cellular debris and the resulting supernatant was centrifuged (130000g, 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 and then freeze-dried.

New protein synthesis was identified by two-dimensional polyacrylamide-gel electrophoresis and autoradiography as previously described (Geheb *et al.*, 1981b). For each pair of fractions

(control and experimental) in a given experiment, equal amounts of radioactive material were subjected to electrophoresis. Exposure times ranged from 5 to 24 days; however, for paired gels, exposure times were always identical. pI and M_r values were determined as previously described (Geheb *et al.*, 1981b).

Results

Comparison of steroid-induced Na^+ transport

The effects of dexamethasone, corticosterone and aldosterone on Na^+ transport were compared over a wide concentration range (1.4 nM to 1.4 μ M) in an extensive series of preliminary experiments (Table 1). The maximal natriferic effect of all three steroids was achieved at the same concentration (~ 140 nM).

Dexamethasone and aldosterone were equipotent over the entire concentration range tested. Approximately half-maximal natriferic responses to aldosterone and dexamethasone were evident at a concentration of ~ 14 nM.

Corticosterone was less natriferic than the other two steroids; at a concentration of 14 nM, Na^+ transport in corticosterone-treated bladders was no different from that in control bladders. At a con-

centration of 1.4 nM, none of the steroids stimulated Na^+ transport.

Effects of spironolactone on steroid-induced Na^+ transport

The effects of spironolactone (SC 9420) on steroid-induced Na^+ transport are shown in Table 2. We have previously demonstrated that an antagonist/agonist ratio of $\sim 2000:1$ is required to inhibit completely aldosterone-induced Na^+ transport (Geheb *et al.*, 1983); consequently, a similar ratio was employed in the present experiments. A significant increase in SCC (relative to control) is evident in the steroid-treated quarter-bladders. Spironolactone completely abolished the natriferic effect of all three steroids without having any effect on basal SCC (between 6 and 10 h). The concentrations of spironolactone (280 μ M) and steroids (140 nM) utilized in these electrophysiological experiments were the same as those subsequently employed in the biochemical studies (see below).

Effects of corticosterone and dexamethasone on new protein synthesis

The effects of maximal natriferic concentrations (140 nM) of corticosterone on new protein synthesis were directly compared with those of aldosterone

Table 1. Comparison of steroid-induced Na^+ transport

In all four series of experiments, both hemibladders (from a single toad) were mounted in modified Ussing chambers to provide four electrically matched quarter-bladders (QB). After a period of stabilization ($\sim 1-3$ h), dexamethasone (Dex), corticosterone (Cort), aldosterone (Aldo) and carrier (methanol) (Con, control) were added to individual quarter-bladders (at 0 h) as indicated. SCC was then continuously monitored for 10 h. SCC values (mean $\pm$ S.E.M.) are provided at 2 h intervals. Statistical comparisons were performed by using Student's *t* test for paired values. Statistical significance: * $P < 0.05$ or better (QB 1, 2 or 3 versus 4). At a concentration of 1.4 nM, none of the steroids were natriferic. At a concentration of 14 nM, dexamethasone and aldosterone were equinatriferic, whereas corticosterone had no effect on Na^+ transport. All three steroids exhibited maximal natriferic activity at a concentration of 140 nM. Note that the time courses of the natriferic responses to different concentrations of the steroids are similar. [The data obtained with aldosterone have been previously published (Geheb *et al.*, 1983).]

Steroid concn.	QB	Additions	Time (h) ...	SCC ($\mu A \cdot cm^{-2}$)					
				0	2	4	6	8	10
1.4 nM ($n = 8$)	1	Dex		17 $\pm$ 2	14 $\pm$ 2	10 $\pm$ 1	7 $\pm$ 1	6 $\pm$ 1	6 $\pm$ 1
	2	Cort		18 $\pm$ 3	15 $\pm$ 3	10 $\pm$ 2	7 $\pm$ 1	6 $\pm$ 1	5 $\pm$ 1
	3	Aldo		19 $\pm$ 3	14 $\pm$ 2	9 $\pm$ 1	6 $\pm$ 1	6 $\pm$ 1	5 $\pm$ 1
	4	Con		18 $\pm$ 2	15 $\pm$ 3	10 $\pm$ 1	6 $\pm$ 1	5 $\pm$ 1	5 $\pm$ 1
14 nM ($n = 8$)	1	Dex		24 $\pm$ 3	20 $\pm$ 4	15 $\pm$ 4	14 $\pm$ 4*	14 $\pm$ 3*	13 $\pm$ 3*
	2	Cort		25 $\pm$ 4	19 $\pm$ 5	14 $\pm$ 5	10 $\pm$ 4	5 $\pm$ 1	6 $\pm$ 1
	3	Aldo		24 $\pm$ 3	19 $\pm$ 5	15 $\pm$ 4	13 $\pm$ 4*	12 $\pm$ 3*	11 $\pm$ 3*
	4	Con		24 $\pm$ 4	20 $\pm$ 5	12 $\pm$ 4	8 $\pm$ 3	4 $\pm$ 1	4 $\pm$ 1
140 nM ($n = 8$)	1	Dex		25 $\pm$ 4	20 $\pm$ 4	17 $\pm$ 3*	16 $\pm$ 3*	17 $\pm$ 3*	15 $\pm$ 2*
	2	Cort		24 $\pm$ 3	20 $\pm$ 3	17 $\pm$ 3*	16 $\pm$ 3*	16 $\pm$ 2*	14 $\pm$ 2*
	3	Aldo		23 $\pm$ 3	19 $\pm$ 2	15 $\pm$ 2*	15 $\pm$ 2*	16 $\pm$ 2*	15 $\pm$ 2*
	4	Con		24 $\pm$ 2	17 $\pm$ 1	10 $\pm$ 1	8 $\pm$ 1	7 $\pm$ 1	6 $\pm$ 1
1.4 μ M ($n = 8$)	1	Dex		25 $\pm$ 3	23 $\pm$ 3	20 $\pm$ 3	17 $\pm$ 2*	16 $\pm$ 2*	12 $\pm$ 2*
	2	Cort		25 $\pm$ 4	24 $\pm$ 3	21 $\pm$ 3	20 $\pm$ 3*	20 $\pm$ 3*	17 $\pm$ 3*
	3	Aldo		25 $\pm$ 4	24 $\pm$ 4	20 $\pm$ 4	19 $\pm$ 4*	18 $\pm$ 3*	16 $\pm$ 3*
	4	Con		25 $\pm$ 4	23 $\pm$ 4	16 $\pm$ 3	10 $\pm$ 2	7 $\pm$ 1	6 $\pm$ 1

Table 2. *Effects of spironolactone on steroid-induced Na⁺ transport*

In all three protocols, both hemibladders (from a single toad) were mounted in modified Ussing chambers to provide four electrically matched quarter-bladders (QB). After a period of stabilization ($\sim 1-3$ h), dexamethasone (Dex), corticosterone (Cort), aldosterone (Aldo), spironolactone (Spiro) and the appropriate carriers (methanol, dimethyl sulphoxide) were added to individual quarter-bladders (at 0 h) as indicated. SCC was then continuously monitored for 10 h. SCC values (mean $\pm$ s.e.m.) are provided at 0, 6 and 10 h after steroid addition. Statistical comparisons were performed by using Student's *t* test for paired values. Statistical significance: **P* < 0.05 or better (QB 1 versus QB 2). The SCC in spironolactone- and spironolactone-plus-steroid-treated quarter-bladders was no different from that in control quarter-bladders. Spironolactone selectively inhibited steroid-induced Na⁺ transport in all three protocols. [The data obtained with aldosterone have been previously published (Geheb *et al.*, 1983).]

Protocol	QB	Additions	Time (h) ...	SCC ($\mu\text{A} \cdot \text{cm}^{-2}$)		
				0	6	10
Dex (<i>n</i> = 5)	1	Dex (140 nM)		42 $\pm$ 6	46 $\pm$ 7*	45 $\pm$ 7*
	2	Control		42 $\pm$ 4	26 $\pm$ 5	12 $\pm$ 2
	3	Dex + Spiro		41 $\pm$ 2	19 $\pm$ 2	12 $\pm$ 2
	4	Spiro (280 μM)		38 $\pm$ 4	18 $\pm$ 2	13 $\pm$ 2
Cort (<i>n</i> = 5)	1	Cort (140 nM)		30 $\pm$ 8	23 $\pm$ 4*	19 $\pm$ 4*
	2	Control		34 $\pm$ 11	14 $\pm$ 3	8 $\pm$ 2
	3	Cort + Spiro		31 $\pm$ 9	11 $\pm$ 2	7 $\pm$ 1
	4	Spiro (280 μM)		27 $\pm$ 6	10 $\pm$ 1	5 $\pm$ 1
Aldo (<i>n</i> = 6)	1	Aldo (140 nM)		40 $\pm$ 10	52 $\pm$ 10*	52 $\pm$ 9*
	2	Control		35 $\pm$ 7	29 $\pm$ 6	18 $\pm$ 6
	3	Aldo + Spiro		40 $\pm$ 8	19 $\pm$ 3	15 $\pm$ 3
	4	Spiro (280 μM)		39 $\pm$ 14	20 $\pm$ 6	13 $\pm$ 3

Fig. 1. *Effects of corticosterone and dexamethasone on new protein synthesis in membrane-rich fractions* Autoradiographs of newly synthesized proteins in membrane-rich fractions (130000g pellet) of epithelial cells were prepared as described in the Methods section. (a, b) Paired hemibladders incubated for 10 h with aldosterone (a) or corticosterone (b); (c, d) paired hemibladders incubated for 10 h with dexamethasone (c) or carrier (d). A maximal natriuretic concentration (140 nM) of all three steroids was employed. Corticosterone (b) and dexamethasone (c) induced the synthesis of proteins (shown in square brackets) with characteristics ($M_r \sim 70000-80000$; $pI \sim 5.5-6.0$) identical with those of proteins induced by aldosterone (a). These proteins are absent from the control hemibladder (d). In this and all subsequent Figures the origin is at the upper left. M_r and pH scales are provided in (a), and for convenience are omitted from (b), (c) and (d) in this Figure and Fig. 2, and altogether in Figs. 3 and 4.

Fig. 2. *Effects of corticosterone and dexamethasone on new protein synthesis in cytosolic fractions* Autoradiographs of newly synthesized proteins in cytosolic fractions (130 000g supernatant) of epithelial cells were prepared as described in the Methods section. (a, b) Paired hemibladders incubated with aldosterone (a) or corticosterone (b); (c, d) paired hemibladders incubated with dexamethasone (c) or carrier (d). A maximal natriferic concentration (140 nM) of all three steroids was employed. Corticosterone (b) and dexamethasone (c) induced the synthesis of a protein with characteristics ($M_r \sim 70\,000$; $pI \sim 5.7$) identical with those of the protein induced by aldosterone (a). This protein is absent from the control hemibladder (d).

in two experiments. In both, corticosterone induced the synthesis of membrane-associated (Fig. 1b) and cytosolic (Fig. 2b) proteins with characteristics identical with those induced by aldosterone (Figs. 1a and 2a). The effect of spironolactone on corticosterone-induced protein synthesis was evaluated in two additional experiments. In both, spironolactone completely blocked the synthesis of the membrane-associated proteins (Figs. 3a and 3b) and markedly inhibited the synthesis of the cytosolic protein (Figs. 4a and 4b).

The effects of maximal natriferic concentrations (140 nM) of dexamethasone on new protein synthesis were examined in four experiments. In all cases, dexamethasone induced the synthesis of membrane-associated (Fig. 1c) and cytosolic (Fig. 2c) proteins with characteristics identical with those induced by aldosterone (Figs. 1a and 2a). The effect of spironolactone on dexamethasone-induced protein synthesis was evaluated in five additional experiments. In all cases, dexamethasone-induced proteins were evident and, in four of the five experiments, spironolactone eliminated (or markedly inhibited) the synthesis of both

the membrane-associated proteins (Figs. 3c and 3d) and the cytosolic protein (Figs. 4c and 4d).

Discussion

Previous studies from our laboratory have suggested that the synthesis of a characteristic group of proteins may be required for expression of aldosterone's natriferic effect in toad urinary bladders (Geheb *et al.*, 1981b, 1983). However, other adrenocortical steroids (often thought of as primarily glucocorticoid in nature) also stimulate Na^+ transport in toad urinary bladders (Edelman, 1968; Handler *et al.*, 1969). In particular, both corticosterone (which may be the endogenous glucocorticoid of the toad) and dexamethasone (a potent synthetic glucocorticoid) are effective natriferic agents (Table 1). Indeed, the natriferic activities of dexamethasone and aldosterone are indistinguishable over the entire concentration range tested: maximal stimulation of Na^+ transport is evident at concentrations of about 100 nM, approximately half-maximal stimulation occurs at about 10 nM, and both steroids are ineffective at concentrations

Fig. 3. Effects of spironolactone on corticosterone- and dexamethasone-induced protein synthesis in membrane-rich fractions

Autoradiographs of newly synthesized proteins in membrane-rich fractions (130 000g pellet) of epithelial cells were prepared as described in the Methods section. (a, b) Paired hemibladders incubated with 140 nM-corticosterone alone (a) or with both corticosterone and 280 μ M-spironolactone (b); (c, d) paired hemibladders incubated with 140 nM-dexamethasone alone (c) or with both dexamethasone and 280 μ M-spironolactone (d). The characteristic membrane-associated proteins (shown in square brackets) are present in the hemibladders treated only with corticosterone or dexamethasone (a and c), but are absent (or markedly decreased) in the hemibladders also treated with spironolactone (b and d).

of about 1 nM. Corticosterone is less natriferic: although maximal activity occurs at concentrations of about 100 nM, at concentrations of 10 nM or less, no enhancement of Na^+ transport is evident. Thus dexamethasone and aldosterone appear to be comparable in their ability to stimulate Na^+ transport in toad urinary bladders and corticosterone appears to be less natriferic than both of these agents.

These data are generally consistent with the relative affinities of the three steroids for the Type I (or putative mineralocorticoid) nuclear receptor (Kusch *et al.*, 1978). Occupancy of this receptor has been correlated with aldosterone's ability to stimulate Na^+ transport in toad urinary bladders (Farman *et al.*, 1978). If dexamethasone and corticosterone stimulate Na^+ transport by a similar mechanism to that of aldosterone, then proteins similar to those induced by aldosterone should be induced by natriferic concentrations of these steroids. Our biochemical studies have demonstrated that this is indeed the case. At concen-

trations of ~ 100 nM, corticosterone and dexamethasone induce proteins with characteristics identical with those induced by aldosterone (Figs. 1 and 2).

Further support for the contention that all natriferic steroids act by similar mechanisms in the toad urinary bladder is provided by the studies with spironolactone. Spirolactones are competitive antagonists of aldosterone-stimulated Na^+ transport, and they have been used to define the mineralocorticoid-specificity of steroid-induced Na^+ transport in renal epithelia (Corval *et al.*, 1981). In toad urinary bladders, spironolactone (SC 9420) displaces [^3H]aldosterone from its cytosolic receptor, and such displacement has been correlated with the ability of spironolactone to inhibit aldosterone-stimulated Na^+ transport (Rossier *et al.*, 1983). We have recently demonstrated that the synthesis of aldosterone-induced proteins in toad urinary bladders is inhibited by spironolactone at the same time that aldosterone-stimulated Na^+ transport is blocked (Geheb *et al.*, 1983). The present data

Fig. 4. Effects of spironolactone on corticosterone- and dexamethasone-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 Methods section. (a, b) Paired hemibladders incubated with 140nM-corticosterone alone (a) or with both corticosterone and 280 μ M-spironolactone (b); (c, d) paired hemibladders incubated with 140nM-dexamethasone alone (c) or with both dexamethasone and 280 μ M-spironolactone (d). The characteristic cytosolic protein (shown in square brackets) is clearly evident in the hemibladders treated only with corticosterone or dexamethasone (a and c) and is markedly decreased in the hemibladders also treated with spironolactone (b and d).

demonstrate that spironolactone also inhibits the natriferic activity of dexamethasone and corticosterone (Table 2). In addition, we have shown that spironolactone inhibits dexamethasone- and corticosterone-induced protein synthesis (Figs. 3 and 4). Thus dexamethasone and corticosterone activate Na^+ transport in toad urinary bladders by similar mechanisms to aldosterone.

It appears, therefore, that steroids with natriferic activity (whether they are 'mineralocorticoids' or 'glucocorticoids') share a common mechanism of action in toad urinary bladders. Indeed, the traditional distinction between these two classes of steroids may not apply in this particular tissue. Natriferic activity can be correlated not only with relative steroid-receptor affinities but also, as we have now demonstrated, with the induction of a characteristic and specific group of proteins. This finding, together with the recent demonstration of similar proteins in cultured kidney cells of the frog *Xenopus laevis* (Blazer-Yost *et al.*, 1982), supports the view that these proteins have an integral role in renal epithelial Na^+ transport.

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