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Aldosterone-induced glycoproteins: electrophysiological-biochemical correlation

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Aldosterone induces the synthesis of a group of glycoproteins (GP65,70) in toad urinary bladders which are potential effectors of the natriferic action of this hormone. In the present study we have confirmed that aldosterone produces a two-phase electrophysiological response. During the early phase (< 3 h) short-circuit current and transepithelial conductance increase in parallel, while during the late phase (> 3 h) short-circuit current continues to increase without any further change in conductance. By biosynthetically labeling aldosterone-treated toad bladders with [^{35}S]methionine either during the early (h 0–2 or 1–3) or the late (h 4–6 or 7–9) phases of the natriferic response, we have demonstrated that GP65,70 is synthesized as a late effect of aldosterone. Since synthesis of GP65,70 occurs at a time when the electromotive force of the Na^+ pump is increasing, and since GP65,70 biochemically resembles the beta subunit of Na^+/K^+-ATPase, studies were undertaken to examine whether GP65,70 is the beta subunit. Purified amphibian renal beta subunit was analyzed by two-dimensional polyacrylamide gel electrophoresis and was found to have an isoelectric point and M_r value similar to those of GP65,70. However, when nitrocellulose blots containing wheat germ agglutinin-purified proteins from aldosterone-treated bladders were stained with monospecific polyclonal antibodies developed against the beta subunit, GP65,70 was not recognized, whereas a group of slightly more acidic proteins of similar M_r were recognized. Thus, GP65,70 is not the beta subunit of Na^+/Ka^+-ATPase. Further studies are needed to determine the cellular function of GP65,70.

Introduction

The natriferic action of aldosterone is mediated by the synthesis of specific proteins which, individually or collectively, modulate the rates of Na^+ entry into, and exit from, renal epithelial cells [1–3]. That aldosterone increases apical cell membrane Na^+ permeability is well established [1,4–8]; this appears to be due to an increase in the number of Na^+ channels rather than to alterations in channel affinity [7]. However, the biochemical mechanisms involved in channel recruitment are unknown.

Since aldosterone-stimulated Na^+ transport is greater than that predicted from the change in transepithelial conductance alone, a hormone-related increase in the electromotive force of the Na^+ pump has been inferred [6,9,10]. Whether this is independent of prior effects of aldosterone on apical Na^+ permeability (and hence of alterations

Abbreviations: TBR, Tris-buffered amphibian Ringer's; MRF, membrane-rich fraction; WGA, wheat germ agglutinin; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis.

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in intracellular Na^+ activity) has long been contested. However, recent studies have demonstrated that, at any given intracellular Na^+ activity, cellular Na^+ extrusion is enhanced by aldosterone pretreatment [8]. Moreover, aldosterone has been shown to increase Na^+/K^+-ATPase synthesis [10,11]; Na^+ pump subunit induction is amiloride insensitive and presumably, therefore, independent of any effects of the hormone on cellular Na^+ entry.

These dual effects of aldosterone (on apical Na^+ conductance and basolateral Na^+ pump activity) predominate at different times during the natriferic response. By comparing the changes in Na^+ transport and transepithelial conductance sequentially throughout the hormone response, it became evident that Na^+ transport and conductance increase in parallel for about 3 h; thereafter, Na^+ transport increases out of proportion to any further change in conductance [6,9,10]. Thus, the 'early' phase of aldosterone's action (up to 3 h after hormone addition) appears to be largely related to changes in apical membrane conductance; any further increase in Na^+ transport after this time ('late' phase) is generally ascribed to an increase in the electromotive force of the Na^+ pump. As with Na^+ channel recruitment, the biochemical mechanisms involved in the modulation of Na^+/K^+-ATPase activity have not been determined. This dual effect of aldosterone on the toad urinary bladder may be subspecies specific, however, since Civan [4] could not demonstrate an increase in Na^+ transport beyond that accounted for by increased apical Na^+ conductance.

Recent studies in our laboratory have raised the possibility that a group of electrophoretically polymorphic (M_r 65000 and 70000) and microheterogeneous (pI 5.8–6.2) glycoproteins (referred to as GP65,70), which are specifically induced by aldosterone (and related natriferic corticosteroids) in toad urinary bladders and A6 cells, represent one of the cellular 'effectors' of aldosterone-stimulated Na^+ transport [12–16]. The induction of these proteins appears to be a direct effect of the hormone (i.e., to be independent of alterations in apical Na^+ permeability). Pretreatment with amiloride does not affect their synthesis [12,13]. Likewise, vasopressin-stimulated Na^+ transport is not associated with the induction of GP65,70 [12].

Although these proteins have been extensively characterized and partially purified, their role in Na^+ transport remains to be defined. The present study was designed to confirm the two-phase response to aldosterone and to relate GP65,70 synthesis to the electrophysiological events occurring at different times during the aldosterone response, events that reflect putative actions of the hormone on the apical and basolateral permeability barriers of the renal epithelial cell. Our results indicate that induction of GP65,70 is not responsible for the early increase in Na^+ conductance. Instead, these proteins appear to be related to the late phase of the natriferic response.

Materials and Methods

Female Dominican toads, *Bufo marinus*, (National Reagents, Bridgeport, CT) were maintained in deionized water for at least 48 h before use. Animals were killed by double pithing and the urinary bladders were excised. Hemibladders were mounted either as paired quarterbladders in modified Ussing chambers (in the electrophysiological studies) or as sacs on glass cannulae (in the biochemical studies). The bladders were bathed in aerated Tris-buffered amphibian Ringers (TBR) which contained (in mM) 5.0 Tris, 0.6 KH_2PO_4, 3.5 Na_2HPO_4, 3.0 KCl, 10.0 dextrose, 0.75 $CaCl_2$ and 5.0 μg/ml gentamicin and 50 U/ml penicillin (pH 7.95).

Stock solutions of aldosterone (Sigma) in methanol ($2.8 \cdot 10^{-3}$ M) were stored at 4°C. [^{35}S]Methionine (spec. act. 1100 Ci/mmol) was purchased from Amersham. Wheat germ agglutinin-Sepharose 6MB (WGA), containing 5 mg lectin/ml gel, was obtained from Sigma and stored at 4°C.

In the electrophysiological studies, after a 1 h period of stabilization, aldosterone was added to the serosal solution of the experimental quarterbladders (final concentration $1.4 \cdot 10^{-7}$ M). The paired control quarterbladders received vehicle alone. The bladders were then continuously clamped at a transepithelial potential of zero for 9 h; short-circuit current (I_{sc}) was used to measure net mucosal to serosal Na^+ flux. At hourly intervals, the open-circuit transepithelial potential difference (V_t) was determined (by briefly interrupt-

ing the short-circuiting) and transepithelial conductance (G_t) was calculated.

To better examine the electrophysiological effects of aldosterone, the absolute changes in I_{sc} and G_t (both designated as x) were corrected (corrected values designated as X) as follows: $X = [(x \text{ at time } 1,2 \ldots n) - (x \text{ at time } 0)]_{aldo} - [(x \text{ at time } 1,2 \ldots n) - (x \text{ at time } 0)]_{con}$. Since starting values for aldosterone-treated and control bladders were similar, this is equivalent to normalization of the data.

In the biochemical studies, hemibladders were mounted as bags and allowed to stabilize for 1 h. Aldosterone ($1.4 \cdot 10^{-7}$ M) was then added to the serosal solutions of all bladders. One bladder of each pair of hemibladders was designated for either 'early' or 'late' radiolabeling of newly synthesized proteins. The 'early' bladders were incubated with [^{35}S]methionine (serosal concentration, 80 μCi/ml) for a 2 h period between either h 0 and 2 or h 1 and 3. The 'late' bladders were incubated with the isotope for a 2 h period between either h 7 and 9 or h 4 and 6.

In the experiments in which GP65,70 was prepared for comparison with the beta subunit of Na^+/K^+-ATPase, bladders were handled as above except that they were incubated with isotope between h 6 and 10. In addition, in these experiments, only one bladder of each pair was treated with aldosterone; the other bladder served as a control.

Immediately following incubation with the isotope, the bladders were placed in iced TBR, and all subsequent preparation occurred at 4°C. Epithelial cells were gently scraped from the mucosal surface of the bladder, pooled, washed three times with iced TBR, and collected by centrifugation ($1200 \times g$, 5 min). A 'membrane-rich fraction' (MRF) was prepared by homogenizing the washed cellular pellet (Dounce homogenizer 40 strokes) in 2 mM EDTA, 220 mM sucrose, 50 mM Tris (pH 7.5). The homogenate was centrifuged ($120\,000 \times g$, 1 h) and the supernatant (cytosol) was discarded. The pellet was solubilized in 1% Triton X-100, 50 mM Tris (pH 8.0) for subsequent purification by wheat germ agglutinin-affinity chromatography.

Wheat germ agglutinin (WGA)-affinity chromatography

WGA-affinity chromatography was accomplished using a batch technique. 2 ml of the lectin preparation were pipetted into polypropylene tubes, 10 volumes of 'prewash' buffer (1% Triton X-100, 50 mM Tris, 150 mM NaCl, 200 mM *N*-acetylglucosamine (GlCNAc) (pH 8.0)) were added, the tube was gently rocked for 15 min and, following centrifugation ($800 \times g$, 5 min) the supernatant was discarded. The gel was then washed with 10 volumes of 'wash' buffer (identical to prewash buffer except for the absence of GlcNAc).

MRF (obtained from six hemibladders) was added to the gel and allowed to bind (with gentle rocking) for 60 min. After centrifugation, the supernatant (nonbound fraction) was decanted and saved. The gel was washed with 25 volumes of 'wash' buffer, by the end of which the last supernatant contained less than 0.5% of the applied radioactivity. Specifically bound protein was eluted by washing three times with 10 volumes of 'elution' buffer (1% Triton X-100, 50 mM Tris, 500 mM GlcNAc (pH 8.0)). The three 'elution' supernatants were combined (bound fraction). This bound fraction was then dialyzed (1200 M_r cutoff) overnight against 1% Triton X-100, 50 mM Tris (pH 8.0), concentrated (ultrafiltration units, Millipore), and precipitated with 10 volumes of cold acetone. Precipitated protein was collected by centrifugation ($12\,000 \times g$, 30 min), air dried, and solubilized in lysis buffer containing 9.5 M urea, 2% Triton X-100, 5% 2-mercaptoethanol, 0.8% Ampholines (LKB) in preparation for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).

2D-PAGE and autoradiography

All specimens were analyzed by 2D-PAGE and autoradiography as previously described [12].

Protein transfer

WGA-purified membrane-rich fractions from aldosterone-treated and control bladders were separated by 2D-PAGE under reducing conditions as described above. The gels were then preequilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol (pH 8.3)) for 30 min, and the protein was transferred electrophoretically to

nitrocellulose membranes (Bio-Rad) under the following conditions: −10 °C, 75 V (constant voltage), 18 h. Following transfer, the blots were dried and autoradiographs were prepared using Kodak X-AR film.

Purified beta subunit of amphibian renal Na^+/K^+-ATPase [17] was dissolved in modified lysis buffer (without 2-mercaptoethanol but containing 1% (v/v) SDS) for a final protein concentration of 0.75 μg/μl. 15 μg aliquots were analyzed by 2D-PAGE using 2-mercaptoethanol-free running buffers. 2-Mercaptoethanol was removed from all solutions in this series of experiments, since it produces a densely stained artifact at M_r 68000 on 2D-PAGE gels [18]. The separated proteins were then either transferred to nitrocellulose membranes as described above or the gels were stained with a sensitive, nickel-based protein stain (Kodavue) obtained from Eastman-Kodak.

Antibody probing

Nitrocellulose blots were incubated for 1 h in 5% instant milk powder in phosphate-buffered saline (blocking solution), and then overnight with monospecific polyclonal antibodies to amphibian renal Na^+/K^+-ATPase [17] diluted 1 : 50 with the same solution. The blots were washed five times with blocking solution containing 0.5% Tween 20 (v/v), and then incubated for 2 h with horseradish peroxidase-conjugated protein A diluted 1 : 1500 with blocking solution. After washing five times in blocking solution, the blots were developed for 5–30 min in 4-chloro-1-naphthol in methanol (3 mg/ml), diluted 1 : 4 with phosphate-buffered saline and containing a final H_2O_2 concentration of 0.01% (v/v). The blots were then washed thoroughly in deionized water and air dried.

Results

Biphasic electrophysiological effects of aldosterone

An effect of aldosterone (10^{-7} M) on I_{sc} was evident between 1 and 2 h; after 2 h, I_{sc} in aldosterone-treated bladders had increased significantly as compared to paired controls ($P < 0.01$, $n = 24$) (Fig. 1). Aldosterone-stimulated I_{sc} rose rapidly between h 1 and 3, and then more slowly (relative to control) for the remainder of the experiment. Transepithelial conductance (G_t) changed in a similar fashion. Treatment with aldosterone led to a rapid increase in G_t beginning about 1 h after hormone addition and reaching a new steady state (which was maintained for the remainder of the experiment) by approximately 3 h (Fig. 2).

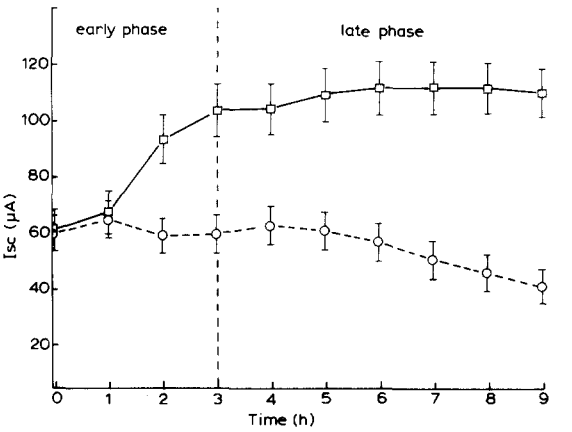

Fig. 1. Short-circuit current (I_{sc}) in paired aldosterone-treated (□) and control (○) quarter-bladders. Aldosterone ($1.3 \cdot 10^{-7}$ M) was added at time zero. The response has two components: an early phase (0–3 h) during which I_{sc} increases rapidly, and a late phase (after 3 h) characterized by a further gradual increase in I_{sc} relative to control. Data are $\bar{X} \pm$ S.E., $n = 24$.

To better compare these electrophysiological

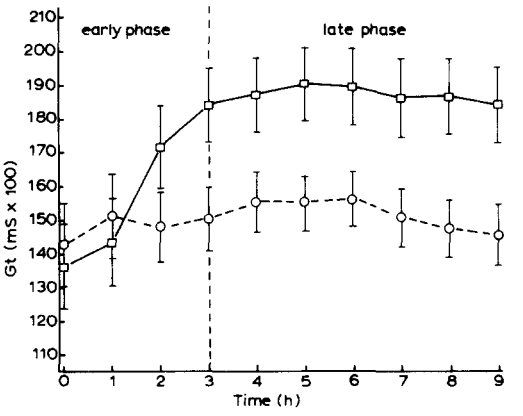

Fig. 2. Transepithelial conductance (G_t) in paired aldosterone-treated (□) and control (○) quarterbladders. Aldosterone ($1.3 \cdot 10^{-7}$ M) was added at time zero. The response has two components: an early phase (0–3 h) during which G_t increases rapidly, and a late phase (after 3 h) where G_t remains relatively constant. Data are $\bar{X} \pm$ S.E., $n = 24$.

effects, the changes in I_{sc} and G_t were normalized as described in Materials and Methods (Fig. 3a). A rapid increase in I_{sc} and a parallel rise in G_t is evident between h 1 and 3. Thereafter, however, I_{sc} continues to increase, while G_t remains stable. Approximately 90% of the overall increase in G_t takes place by h 3, whereas only about 60% of the overall increase in I_{sc} has occurred by this time. Thus, 'early' and 'late' effects of aldosterone on Na^+ transport can be demonstrated. A direct comparison of the changes in I_{sc} and G_t at hourly intervals during the time course of the aldosterone response most dramatically reveals these two phases (Fig. 3b). During the early phase of aldosterone's action (up to 3 h) the increase in Na^+ transport is paralleled by a similar increase in G_t, while during the late phase (after 3 h) the rise in Na^+ transport is independent of any further changes in G_t.

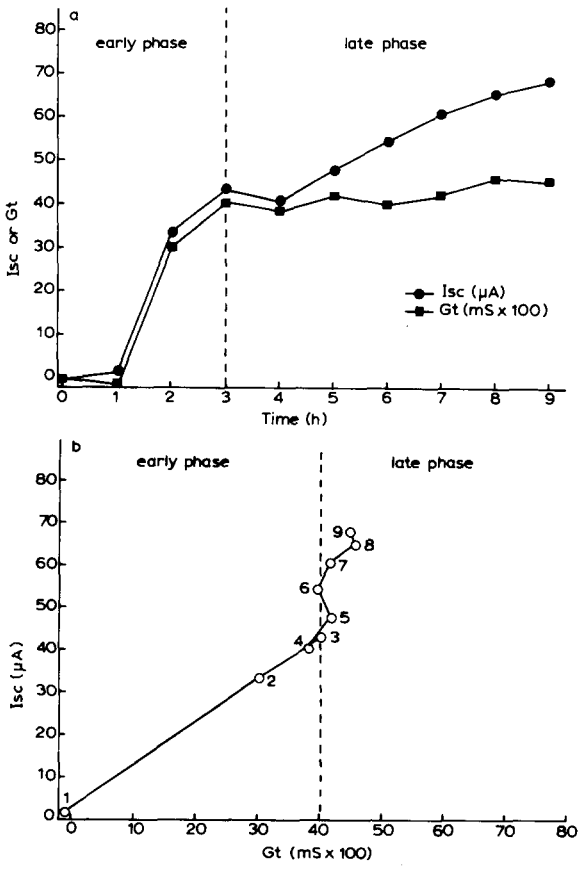

Fig. 3. (a) Relationship between I_{sc} (μA) and G_t (mS$\times$100) at hourly intervals for 9 h following incubation with aldosterone corrected for both starting and control values (see Materials and Methods). The vertical dotted line separates the early (0–3 hr) from the late (3–9 h) response. The early response is characterized by a parallel increase in I_{sc} and G_t. During the late response, I_{sc} continues to increase despite little further change in G_t. The continued rise in I_{sc} after 3 h represents an increase in the electromotive force of the sodium pump (E_{Na^+}). (b) I_{sc} (μA) and G_t (mS$\times$100) corrected for both starting and control values are plotted together to better illustrate the two-phase nature of the aldosterone response. From 0 to 3 h, I_{sc} and G_t increase in parallel (early phase). From 3 to 9 h I_{sc} continues to increase while G_t remains stable (late phase). Data for (a) and (b) are $\bar{X}$, $n = 24$; S.E. are omitted for purposes of clarity. Normalization was performed as described in Materials and Methods.

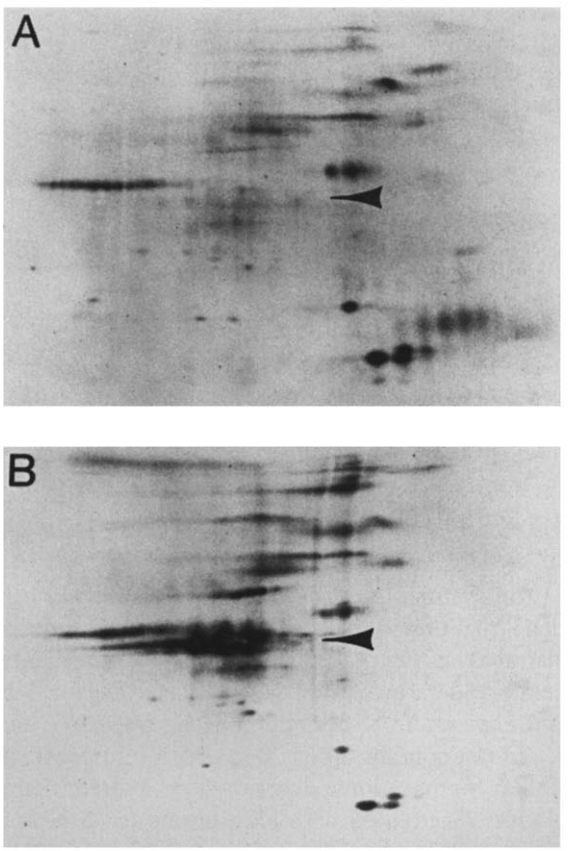

Fig. 4. Time course of the induction of GP65,70 by aldosterone in toad urinary bladders. The autoradiograph in panel A was obtained from wheat germ agglutinin-purified epithelial cell proteins biosynthetically labeled during h 1–3 after the addition of aldosterone (10^{-7} M), while the proteins in panel B were labeled during h 4–6 after hormone treatment. As is illustrated, GP65,70 (arrow) is induced as a late effect (after 3 h) of aldosterone.

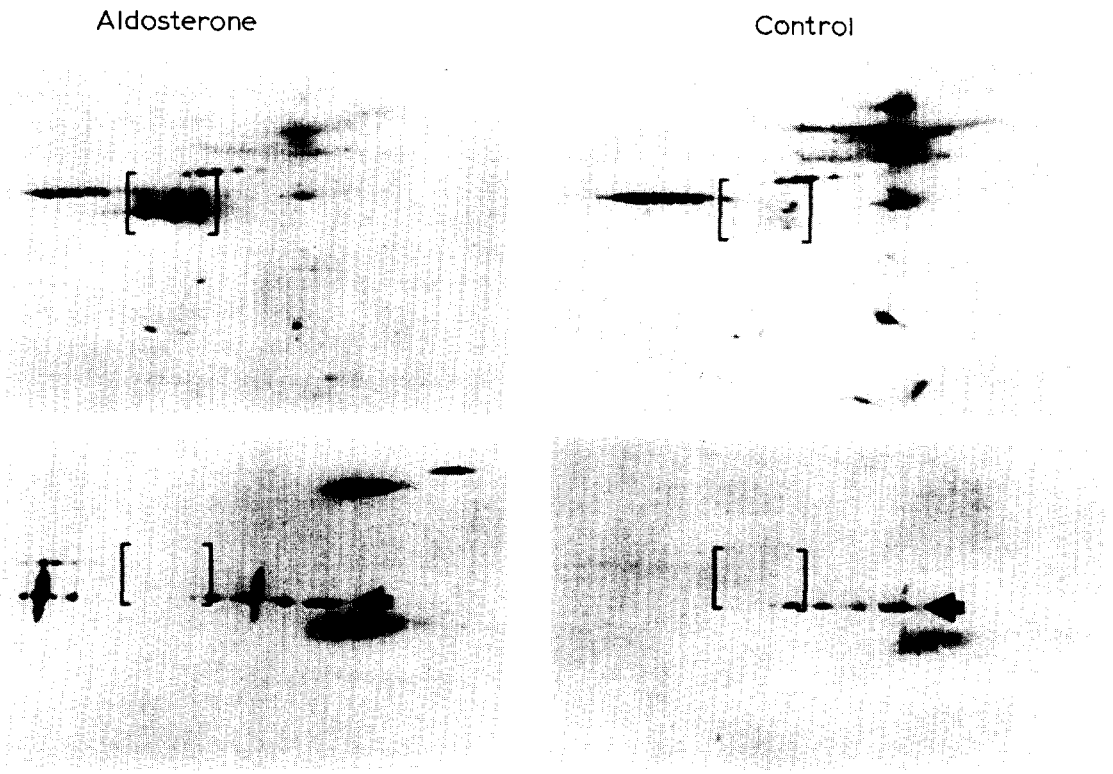

Fig. 5. Upper panels: autoradiographs of nitrocellulose blots prepared from 2D-PAGE gels of wheat germ agglutinin-purified epithelial cell proteins derived from paired aldosterone-treated or control toad urinary bladders. GP65,70 [brackets] is virtually absent from the control tissue. Lower panels: corresponding blots after immunostaining using monospecific polyclonal antibodies to the beta subunit of toad kidney Na^+/K^+-ATPase. A group of proteins of the same M_r as GP65,70, but of more acidic p*I*, is specifically recognized by the antibody (arrow). GP65,70 is not recognized.

Relationship of GP65,70 synthesis to the biphasic effects of aldosterone

Autoradiographs of WGA-purified membrane-rich fractions, separated by 2D-PAGE, are illustrated in Fig. 4. In two separate experiments, hemibladders incubated with aldosterone for 3 h, and exposed to [^{35}S]methionine between h 1 and 3, did not contain GP65,70 (panel A). In contrast, similar preparations derived from paired hemibladders incubated with aldosterone for 6 h, and exposed to isotope between h 4 and 6, revealed the characteristic GP65,70 complex (panel B). Similar findings were obtained in three additional experiments in which paired hemibladders were incubated with aldosterone for 2 h and exposed to isotope between h 0 and 2, or incubated with aldosterone for 9 h and exposed to isotope between h 7 and 9 (data not shown). Thus, the GP65,70 complex is synthesized only during the late phase (after 3 h) of aldosterone-stimulated Na^+ transport.

Although the primary objective of this study was to correlate the synthesis of one particular group of aldosterone-induced proteins (GP65,70) with the electrophysiological effects of the hormone, we also examined whether there were any other proteins whose synthesis could be localized to one particular phase of the aldosterone response. However, in the five experiments performed, no other reproducible time-dependent effects were observed.

Comparison of GP65,70 to the beta subunit of Na^+/K^+-ATPase

To examine whether GP65,70 represents the beta subunit of Na^+/K^+-ATPase, WGA-purified

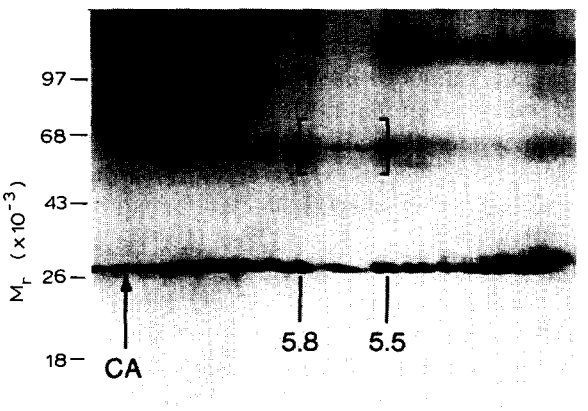

Fig. 6. Kodavue-stained 2D-PAGE gel of immunoaffinity-purified beta subunit of toad kidney Na^+/K^+-ATPase. Carbamylated carbonic anhydrase is included as an internal p*I* standard. Brackets surround the predominant stained protein (M_r 65000, p*I* 5.6–5.8).

membrane-rich fractions from aldosterone-treated and control bladders were electrophoretically transferred from 2D-PAGE gels to nitrocellulose. These 'blots' were then probed with monospecific polyclonal antibodies to amphibian renal beta subunit. As shown in Fig. 5, GP65,70 is not recognized. Interestingly, a microheterogeneous group of slightly more acidic proteins of similar M_r to GP65,70 were revealed in 'blots' prepared from both aldosterone-treated and control bladders. In all likelihood these represent the beta subunit of Na^+/K^+-ATPase, since (1) purified beta subunit has a very similar, if not identical appearance in Kodavue-stained 2D-PAGE gels (Fig. 6), and (2) monospecific anti-beta antibodies recognize the same group of proteins on Western blots of 2D-PAGE gels containing the purified beta subunit (data not shown). Staining of other proteins on the gel in Fig. 5 probably represent cross reactivity of the antibody preparation with other glycoproteins.

Discussion

Transepithelial conductance (G_t) is a complex term that includes paracellular (shunt) conductance as well as apical and basolateral plasma membrane conductances. However, the major barrier to Na^+ movement in the toad urinary bladder is the apical membrane and aldosterone has a major (although not exclusive) effect on G_t by way of increasing apical membrane conductance [1,4–8]. Consequently, changes in G_t can reasonably be considered an approximation of alterations in apical membrane conductance. Similarly, E_{Na^+} (as measured in the present study) should be considered an approximation of the true Na^+ pump potential, depending not only on the thermodynamics of the Na^+/K^+-ATPase but also on a variety of other electrodiffusive processes.

The present study confirms that aldosterone produces a two phase electrophysiological response in toad urinary bladders – an 'early' phase (h 1–3) during which I_{sc} and G_t increase in parallel, and a 'late' phase (after 3 h) during which I_{sc} continues to increase in the absence of any further change in G_t. The early phase is likely related to recruitment of apical membrane Na^+ channels – either by activation of pre-existing channels or by insertion of additional channels from a preformed pool [7]. Presently available evidence, although limited in scope, suggests that aldosterone activates Na^+ channels already present in the apical cell membrane [19,20].

Since GP65,70 is not synthesized during the early phase of the hormone's action, it is unlikely that these proteins are responsible for the initial increase in transepithelial (presumably, largely apical membrane) conductance. Consequently, a separate, and so-far uncharacterized, aldosterone-induced protein (or group of proteins) must account for the effects of the hormone on Na^+ channel function. These conclusions are, of course, subject to the caveat that small amounts of GP65,70 (undetectable even in WGA-purified membrane-rich fractions) could have a relatively large effect on Na^+ transport.

The late phase of aldosterone's action is thought to reflect hormonal modulation of Na^+ pump function – an increase in the number of pumps, an increase in the electromotive force of the pump (E_{Na^+}), or both. Recent evidence has demonstrated that for any given intracellular Na^+ activity, basolateral extrusion of Na^+ is enhanced by aldosterone [8]. In addition, biochemical studies, using monospecific polyclonal antibodies to amphibian Na^+/K^+-ATPase, have shown that aldosterone increases the synthesis of both the alpha

and the beta subunits of the Na^+ pump in toad urinary bladders [10,11]. This increase only becomes detectable after 6 h incubation with the hormone. Na^+ pump subunit synthesis is amiloride insensitive and presumably, therefore, independent of prior effects of aldosterone on apical membrane Na^+ permeability.

Whether these newly synthesized subunits are assembled and inserted into the basolateral cell membrane and function as active Na^+ pumps has not been determined. Although rabbit cortical collecting tubule Na^+/K^+-ATPase activity is increased by aldosterone [21], and membrane preparations from aldosterone-treated A6 cells exhibit more ouabain binding than do control preparations [22], both of these events are amiloride sensitive; consequently, they probably reflect increased intracellular Na^+ activity rather than a direct effect of aldosterone on the Na^+ pump. Thus, in contrast to pump subunit synthesis (where a direct effect of aldosterone is reasonably well established). Na^+ pump expression (assembly, insertion, activation) may be dependent, at least in part, on prior effects of the hormone on apical membrane Na^+ permeability.

Since GP65,70 is synthesized at a time when induction of Na^+ pump subunits has been demonstrated [10,11], the possibility that these proteins represent a component of the Na^+/K^+-ATPase (specifically the beta subunit, which is a glycoprotein of similar molecular weight to GP65,60) was explored. Analysis of purified toad kidney beta subunit by 2D-PAGE revealed a microheterogeneous group of proteins of p*I* and M_r values closely similar to those GP65,70. However, GP65,70 was not recognized on Western blots by monospecific polyclonal antibodies to the beta subunit. Thus, GP65,70 appears to be antigenically distinct from the beta subunit.

It is of interest that the anti-beta subunit antibodies did recognize a group of proteins (of similar M_r, but with slightly more acidic p*I* compared to GP65,70) in both aldosterone-treated and control bladders (Fig. 5). The similar degree of immunostaining of the presumptive beta subunit in both aldosterone-treated and control bladders should not be taken as evidence that beta subunit synthesis is insensitive to aldosterone. Not only is there considerable evidence to the contrary [10,11], but it should also be stressed that immunostaining on blots prepared from the total cellular pool of proteins cannot be used to assess rates of synthesis of individual proteins. Moreover, 2D-PAGE is not a quantitative technique. The demonstration that aldosterone increases beta subunit synthesis was obtained using specific immunoprecipitation of pulse-labeled Na^+/K^+-ATPase [10,11], a quantitative technique less subject to errors arising from incomplete solubilization of membrane proteins and non-penetration of such proteins into the first dimension of 2D-PAGE gel systems.

What, then, is the nature and function of GP65,70 as a late-phase aldosterone-induced protein? Within the context of the Ussing-Skou 'two barrier' model of epithelial Na^+ transport, possible candidates would include: (a) a modulator of Na^+/K^+-ATPase pump activity or (b) a component of the apical Na^+ channel. With regard to the first possibility, it should be recalled that an increase in the number of Na^+ pumps alone should not result in an increase in E_{Na^+}. Consequently, induction of Na^+/K^+-ATPase per se is unlikely to be a complete explanation of the pump-related events occurring during the late phase of aldosterone-stimulated Na^+ transport. However, the existence of such 'pump modulating' proteins remains speculative at the present time.

With regard to the second possibility, it may, at first sight, seem unlikely that GP65,70 could be a component of the apical Na^+ channel. After all, G_t is essentially stable during the late phase of the aldosterone response; consequently, any newly synthesized channels would appear to be inactive, and therefore such synthesis could appear to serve no useful purpose. However, it can be argued that channels synthesized during the late phase of the response replace pre-existing channels whose functional lifespan has been exceeded during the prolonged natriferic response to aldosterone. Such synthesis would then be a prerequisite for the maintenance of apical Na^+ permeability at a level sufficient to support an enhanced rate of Na^+ transport over the entire time-course of aldosterone's action.

In this schema, aldosterone is considered to have two distinct, but interrelated, actions on the apical permeability barrier: (a) an early effect involving activation of pre-formed Na^+ channels

(characterized by a rapid increase in G_t) [19,20] and (b) a late effect involving replacement of previously activated Na^+ channels with newly synthesized, active channels (characterized by maintenance of the enhanced G_t). Until the renal epithelial apical Na^+ channel is isolated and characterized such a hypothesis must, of course, remain speculative. However, it is of interest that initial work in this regard indicates that one of the components of the channel has a M_r very similar to that of GP65,70 [23,24].

In summary, the present studies have shown that GP65,70 synthesis is restricted to the 'late' portion of the natriferic response to aldosterone. In addition, we have shown that GP65,70 is not the beta subunit of the Na^+/K^+-ATPase pump. GP65,70 could serve as a modulator of basolateral Na^+ pump activity. Alternatively, GP65,70 may represent 'late' synthesis of a component of the apical Na^+ channel. One way to distinguish between these two possibilities would be to localize GP65,70 to either the apical or the basolateral cell membrane, a project which is currently underway in our laboratory using monoclonal antibodies to GP65,70 [25].

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