Mechanisms of inactivation of the action of aldosterone on collecting duct by TGF-β

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Husted, Russell F., Rita D. Sigmund, and John B. Stokes. Mechanisms of inactivation of the action of aldosterone on collecting duct by TGF-β. Am. J. Physiol. Renal Physiol. 278: F425–F433, 2000.—The purpose of these experiments was to investigate the mechanisms whereby transforming growth factor-β (TGF-β) antagonizes the action of adrenocorticoid hormones on Na+ transport by the rat inner medullary collecting duct in primary culture. Steroid hormones 1) increased Na+ transport by three- to fourfold, 2) increased the maximum capacity of the Na+–K+ pump by 30–50%, and 3) increased the steady-state levels of the α subunit of the Na+–K+–ATPase by ~30% and 4) increased the steady-state levels of the α subunit of the rat epithelial Na+ channel (α-rENaC) by nearly fourfold. TGF-β blocked the effects of steroids on the Na+ transport and the stimulation of the Na+–K+–ATPase and pump capacity. However, there was no effect of TGF-β on the steroid-induced increase in α-rENaC mRNA. The effects of TGF-β were not secondary to the decrease in Na+ transport per se, inasmuch as benzamil inhibited the increase in Na+ transport but did not block the increase in pump capacity or Na+–K+–ATPase mRNA. The results indicate that TGF-β does not inactivate the steroid receptor or its translocation to the nucleus. Rather, they indicate complex pathways involving interruption of the enhancement of pump activity and activation/inactivation of pathways distal to the steroid-induced increase in the transcription of α-rENaC.

sodium transport; inner medullary collecting duct; epithelial sodium channel; sodium-potassium-adenosine triphosphatase; benzamil; glucocorticoid; mineralcorticoid; Northern blot; ribonuclease protection assay; electrophysiology

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The Molecular Actions of Steroid Hormones that Produce an Increase in Electrogenic Na+ Transport by High-Resistance Epithelia Remain Poorly Understood, Despite Extensive Investigation over Several Decades. Recently, We Reported that Transforming Growth Factor-β (TGF-β) Can Render the Rat Inner Medullary Collecting Duct (IMCD) Unresponsive to the Natriferic Effects of Mineralcorticoid and Glucocorticoid Hormones (13, 14). The Unique Features of This Action of TGF-β Include 1) No Effect on Na+ Transport Within 30 Min of TGF-β Exposure, 2) No Effect on the Capacity for Cl− Secretion in Response to CAMP, and 3) Prolonged Resistance to Steroids for >2 Days after Removal of TGF-β from the Media.

Increasing evidence points to the possible physiological relevance of the effects of TGF-β on IMCD function in vivo. First, there is a considerable amount of TGF-β in the inner medulla normally. The amount of TGF-β mRNA is much higher in the inner medulla than in the cortex, as assessed by immunocytochemistry (24). Second, recent evidence indicates that dietary NaCl can regulate the amount of TGF-β in the kidney: a high-salt diet increases TGF-β production and excretion (42). Finally, TGF-β production is increased in several disorders of renal function. One of the best studied is obstructive uropathy, where acute and chronic obstruction produces time-dependent and regional changes in TGF-β (6, 21, 24). This increased production of TGF-β may play a role in the well-described phenomenon of aldosterone resistance in some cases of urinary obstruction.

These results suggest that TGF-β can antagonize the actions of aldosterone in vivo. This interaction may play an important role in the low level of Na+ absorption by IMCD dissected from rodents treated with mineralocorticoid hormone (16, 25, 30). The reason for the relatively high amounts of TGF-β in the inner medulla is not clear. One possibility is to down-regulate the actions of aldosterone on the IMCD in the salt-replete state. In this regard, it is important to note that a major difference between the cortical collecting duct (CCD), where there is little TGF-β, and the IMCD is the capacity for K+ secretion. Whereas the CCD responds to aldosterone by secreting large amounts of K+, the IMCD does not possess an intrinsic capacity for active K+ secretion (31). It seems possible that varying the responsiveness of the collecting duct to aldosterone within regions of the kidney might effect regional differences in Na+ absorption. Thus TGF-β might play a role in regulating Na+ balance by modulating regional responsiveness to aldosterone.

The present studies were conducted to gain insight into the actions produced by aldosterone and the mechanisms whereby TGF-β antagonizes these actions. We focused on the apical membrane entry pathway, the epithelial Na+ channel (ENaC), and the basolateral Na+–K+ pump, because these molecules play the dominant role in regulating the steroid-induced effects (34). We conducted the studies in three phases: 1) the actions...
of steroids alone, 2) the actions of steroids on IMCD cells, where Na⁺ entry was inhibited with the amiloride analog benzamil, and 3) the action of aldosterone in the presence of TGF-β. The results provide insights into molecular mechanisms whereby Na⁺ transport by the collecting duct might be regulated.

**METHODS**

Preparation of monolayers. Primary cultures of IMCD cells were prepared from 4- to 5-wk-old Wistar rats by the hypotonic lysis isolation method, as previously described by this laboratory (11, 12, 18). The inner medulla was dissected, minced, and incubated in an isotonic solution containing 0.1% collagenase for 2–3 h. The solution was made hypotonic by addition of two volumes of distilled water containing 10 µg/ml DNase, and cells were recovered after two centrifugation steps. This isolation procedure usually yielded 20–40 12-mm monolayers from 6 kidneys.

Cells were seeded onto collagen-coated Millicell PCF filters (Millipore). Seeding density was 20 µg DNA/12-mm filter or 140 µg DNA/30-mm filter (~350,000 cells/cm²). Cells were grown for 3 days in a medium composed of a 1:1 mixture of DMEM-Ham’s F-12 supplemented with 100 µg/ml gentamicin, 20 µg/ml norfloxacin, 5 mM triiodothyronine, 50 mM cortisol, 5 µg/ml transferrin, 5 µg/ml bovine insulin, 10 mM sodium selenite, and 1% (wt/vol) bovine albumin. On the 3rd day the medium was changed to one from which cortisol, norfloxacin, and albumin were omitted. After 24 h in the steroid-free medium, the monolayers were confluent, as evidenced by a transmonolayer electrical resistance (R₊), of >100 Ohm/cm². Where indicated, each isolation was randomized to a treatment group by a Latin square procedure according to the short-circuit current (Iₛₑ). Monolayers were exposed to 100 nM dexamethasone and 10 µM spironolactone, a mineralocorticoid receptor antagonist (GC), 100 nM aldosterone and 10 µM RU-38486, a glucocorticoid receptor antagonist (27) (MC), or vehicle (control, ethanol) for 24 h. These treatments provide nearly complete occupancy of the glucocorticoid and mineralocorticoid receptors with minimal crossover occupancy (12).

Electrical measurements. R₊ and Iₛₑ were initially measured under sterile conditions by placement of 12-mm Millicell filters into modified Ussing chambers (J. Im’s Instruments, Iowa City, IA). Measurements were made in media without additives at 37°C with a University of Iowa voltage clamp (11, 12, 18). A positive Iₛₑ indicates a flow of positive charges from the apical to the basal surface. Electrical measurements for pump current were made in nonsterile chambers designed to accommodate Millicell PCF filters. Amphoterin C B (30 µM) was added to the apical solution to permeabilize the apical membrane to monovalent ions (15). The solution used to measure pump current contained (in mM) 25 NaCl, 120 tetramethylammonium monohydrate, 90 gluconic acid, 5 sodium HEPES, 5 acid HEPES, 1.5 CaCl₂, 1.0 MgCl₂, 2 BaCl₂, and 5 D-glucose, pH 7.4. After steady state was achieved, ouabain (2 mM) was added to the basolateral solution. The ouabain-sensitive current was taken as a measure of the maximum capacity of the pump to transport Na⁺, as previously described (15).

Northern blot analysis of the α₁ and β₁-subunits of the Na⁺-K⁺-ATPase. Total RNA was prepared from IMCD monolayers grown on 30-mm filters by the acid guanidinium thiocyanate-phenol-chloroform extraction method, as described previously (4, 37). Each 30-mm filter yielded ~20–25 µg of RNA. This RNA was dissolved in water, denatured, and subjected to electrophoresis through a 1.5% agarose-6% formaldehyde gel at 100 V for 2 h at 18°C. The RNA was transferred by capillary electrophoresis to Hybond N nylon membrane (Amersham, Arlington Heights, IL) and cross-linked (UV Stratalinker, Stratagene, La Jolla, CA).

The rat α₁ and β₁ cDNA clones were a kind gift from Jerry Lingrel (29). We used actin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to normalize for loading. We also determined the loading by densitometry of the ribosomal bands on the agarose gel by ethidium bromide staining. None of the treatments affected GAPDH or actin mRNA abundance. The cDNA was excised from the cloning vector by use of the appropriate restriction endonuclease and gel purified using Geneclean (Bio 101, Vista, CA). The probes were radiolabeled with [α-32P]dCTP (3,000 Ci/mmol) with use of a random prime DNA labeling kit (Boehringer-Mannheim). The hybridization procedure proceeded as described previously (5, 37). After hybridization, the bands were quantitated using scanning densitometry with Quantity One software (PDI, Huntington Station, NY). The exposure of the autoradiograms was adjusted so that the density of each of the bands fell into the linear range of the instrument.

**RNase protection assay.** The constructs used for the RNase protection assay (RPA) were those previously described for α-, β-, and γ-subunits of rat ENaC (rENaC) and for GAPDH (37). The α-rENaC construct was a 422-nt segment, the β-rENaC construct was a 249-nt segment, and the γ-rENaC construct was a 190-nt segment. The γ-rENaC construct was shortened from that previously reported (37) by using the BsrF1 restriction site. The rat GAPDH construct was a 140-nt segment extending from the translation start site to the first StyI restriction site. All the probes represented unique sequences directed against segments within the open reading frame.

Antisense probes for the RPA were synthesized from the appropriate constructs with the BrightStar BIOTINscript nonisotopic in vitro transcription kit (Ambion, Austin, TX). The amount of biotin-labeled CTP was adjusted to give the highest possible specific activity. The lengths of the biotin-labeled, unprotected products were 480, 280, 453, and 220 nt for α-, β-, and γ-rENaC and GAPDH, respectively.

The hybridization of ~1 ng of each of these probes with RNA from a single 30-mm filter (~25 µg total RNA) was conducted using the RPAII RPA kit (Ambion). After RNase treatment, the products were subjected to electrophoresis through a 5% denaturing polyacrylamide-8 M urea gel buffered with Tris borate for 2.5 h at 250 V and transferred to a nylon membrane (BrightStar Plus, Ambion) with use of a semidry electroblotter (Fisher, Itasca, IL). The membrane was subsequently ultraviolet cross-linked (UV Stratalinker, Stratagene), and the protected RNA fragments were developed using the BrightStar Bioblot detect nonisotopic detection kit (Ambion) with minor modifications. To reduce background, the wash times after incubation with the streptavidin-alkaline phosphatase conjugate solution were increased threefold. The developed blots were exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) for 1–45 min, depending on the intensity.

The α-rENaC probe occasionally showed degradation products when the amount of the respective mRNA was large. The magnitude of these products was <10% of the completely protected fragment, and the shorter fragments did not interfere with the ability to quantitate any of the other bands of interest. Therefore, all quantitation was conducted on the major protected fragments.

Statistics. Data were analyzed by paired analysis or by ANOVA, as indicated. When data were found to be inhomogeneous by Bartlett’s test, analyses were conducted on logarithm-
the basolateral membrane Na\textsuperscript{+} pump. It is clear that steroids increased the transcellular Na\textsuperscript{+} transport to a greater extent than they increased the maximum capacity of the pump. Therefore, steroids increased the fraction of the maximal capacity at which the pump functioned from 21% to ~50% (Fig. 1C). The effect of GC was not different from that of MC. These results are similar to those we reported for IMCD monolayers isolated from Dahl salt-sensitive and -resistant rats (15) and imply that steroids increase the entry of Na\textsuperscript{+} across the apical membrane to a greater extent than they increase the capacity of the Na\textsuperscript{+} pump.

We measured the effect of GC and MC on the mRNA abundance of the α₁- and β₁-subunits of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase by Northern blot analysis. Figure 2 shows an example of a series where MC and GC effects were examined at 3, 8, and 24 h. Inasmuch as the maximal effect of steroids on Na\textsuperscript{+} transport and mRNA abundance seemed to occur at ~24 h, we continued the analyses using this time point. As shown in Fig. 3, both steroid treatments produced a modest (27–39%) but significant increase in the abundance of the α₁-subunit of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA. There was a tendency for steroid treatment to increase the abundance of the β₁-subunit of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA, but the effect was not statistically significant.

Interaction of steroids and blockade of Na\textsuperscript{+} entry. In analyzing the action of an agent that inhibits Na\textsuperscript{+} transport on the intermediate steps leading to alteration of transport, it is important to assess the effects of steroids under conditions where the increase in the rate of Na\textsuperscript{+} transport is prevented by other means. Hence, we examined the effect of steroids on IMCD monolayers that had been treated with 1 µM benzamil to inhibit Na\textsuperscript{+} transport.

Figure 4A shows the effect of 24 h of benzamil treatment with and without steroid treatment. In the absence of benzamil, GC and MC had their usual stimulatory effect on Na\textsuperscript{+} transport. Benzamil produced a reduction in I
\textsubscript{sc} in all groups (~80%). The magnitude of I
\textsubscript{sc} is larger in monolayers treated with steroids and benzamil than in monolayers treated with benzamil alone. We are not certain of the reason for this difference. We intentionally used a low concentration of benzamil (1 µM) to reduce the possibility of benzamil producing effects other than inhibition of the Na\textsuperscript{+} channels. It is possible that benzamil may be partially metabolized by these cells over the 24 h of treatment and thus may have less active product available for

**RESULTS**

Effect of steroids on Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. Figure 1 shows the effect of GC or MC treatment for 24 h on the Na\textsuperscript{+} transport rates and maximum pump capacity. Steroids increased I
\textsubscript{sc}, a measure of Na\textsuperscript{+} transport by intact monolayers (12, 18), by 3.3-fold (Fig. 1A). Steroids also increased the maximum capacity of the Na\textsuperscript{+} pump by 30–50% (Fig. 1B). In the steady state the rate of Na\textsuperscript{+} transport across the apical membrane (through the Na\textsuperscript{+} channel) must be equal to the rate of exit through the basolateral membrane Na\textsuperscript{+} pump. It is clear that steroids increased the transcellular Na\textsuperscript{+} transport to a greater extent than they increased the maximum capacity of the pump. Therefore, steroids increased the fraction of the maximal capacity at which the pump functioned from 21% to ~50% (Fig. 1C). The effect of GC was not different from that of MC. These results are similar to those we reported for IMCD monolayers isolated from Dahl salt-sensitive and -resistant rats (15) and imply that steroids increase the entry of Na\textsuperscript{+} across the apical membrane to a greater extent than they increase the capacity of the Na\textsuperscript{+} pump.

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inhibition of the Na\textsubscript{1} channel.\textsuperscript{1} We think it unlikely that steroids are stimulating Cl\textsubscript{2} secretion, which would produce a benzamil-insensitive current, inasmuch as we previously showed that steroids do not enhance this current in IMCD cells (14). In any event, treatment with benzamil produced what we had planned: a large reduction in the magnitude of Na\textsubscript{1} transport in steroid-treated monolayers.

Figure 4 shows the effect of these treatments on the magnitude of the pump current. There are three points. First, benzamil did not prevent steroids from stimulating the pump current (\(\# P < 0.0001\)). There was no difference in the ability of MC or GC to stimulate pump current with or without benzamil. Second, benzamil modestly reduced the maximum pump current (by \(\sim 14\%\)) irrespective of whether the monolayers were treated with steroids (\(P < 0.01\)). Third, there was no interaction between steroids and benzamil by two-way ANOVA. Thus benzamil had a small inhibitory effect on maximum pump current but did not interfere with the ability of steroids to produce stimulation.

The effect of benzamil on the abundance of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunit mRNA is shown in Fig. 5. In general, benzamil had no effect on the abundance of the \(\alpha_1\) and \(\beta_1\)-subunits of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA. However, there was a single exception. Benzamil increased the abundance of the \(\alpha_1\)-subunit of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA in monolayers treated with MC, but not in those treated with GC. The same tendency was seen for the \(\beta_1\)-subunit of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA, but the effect did not reach statistical significance. These results suggest that the increase in the maximum capacity of the pump produced by steroids is not secondary to an increase in Na\textsuperscript{+} transport. This effect appears to be a direct steroid effect.

Interaction of TGF-\(\beta_1\) and steroid treatment of IMCD monolayers. Figure 6 shows the effect of TGF-\(\beta_1\) on Na\textsuperscript{+} transport by intact IMCD monolayers. When monolayers were treated with MC and TGF-\(\beta_1\) together, the increase in Na\textsuperscript{+} transport produced by MC was largely prevented (Fig. 6A). This result is similar to that which

\textsuperscript{1}We tested this hypothesis on M1 cells grown under similar conditions. In this cell line, long-term (24-h) incubation with benzamil is less effective in reducing Na\textsuperscript{+} transport than it is in IMCD cells. Benzamil at 1 \(\mu M\) produced only 22\% of the inhibitory capacity of fresh benzamil (1 \(\mu M\)) applied to the same monolayers. It appears that collecting duct cells may have a capacity to metabolize benzamil.
we previously reported (13). We did not study the effect of TGF-β1 in monolayers not treated with steroids (in this group of experiments), inasmuch as the effect is small or negligible (13).

As shown in Fig. 6B, whereas MC stimulated maximum pump current by ~30%, monolayers treated with both MC and TGF-β1 had pump currents not different from those without steroid. The ratio of the Isc to the maximum capacity, the fraction of the maximal capacity at which the pump operates in intact monolayers (Fig. 6C), was increased by MC, and the increase was eliminated by TGF-β1.

The effect of steroid and TGF-β1 treatments on Na⁺-K⁺-ATPase subunit abundance is shown in Fig. 7. We combined the results of experiments with GC or MC treatments, inasmuch as there were no differences. Steroid treatment increased the abundance of the α1-subunit of Na⁺-K⁺-ATPase mRNA by 33%, whereas there was no effect on the abundance of the β1-subunit of Na⁺-K⁺-ATPase mRNA (similar to Fig. 3). TGF-β1 in the absence of steroid had no effect on the abundance of either subunit. However, TGF-β1 prevented the steroid-induced increase in the abundance of the α1-subunit of Na⁺-K⁺-ATPase mRNA.

The effect of MC and TGF-β1 on rENaC mRNA abundance is shown in Figs. 8 and 9. As we showed previously using a Northern blot analysis (37), MC increased the abundance of α1-rENaC mRNA but had no effect on β- or γ-rENaC mRNA. TGF-β1 in the absence of steroid had no effect on the abundance of the mRNA of any of the subunits. In contrast to its effect on the α1-subunit of Na⁺-K⁺-ATPase mRNA, TGF-β1 had no effect on the ability of MC to increase α1-rENaC mRNA abundance.

**DISCUSSION**

The present results begin to address the mechanisms involved in the resistance of distal tubular epithelia to the action of steroid hormones. To determine how steroid hormone action might be antagonized, we first needed to establish the actions of steroid hormone on primary cultures of IMCD cells. Steroids increase Na⁺ transport by 1) increasing the maximum capacity of the Na⁺-K⁺ pump (Fig. 1), 2) increasing the abundance of the α1-subunit of Na⁺-K⁺-ATPase mRNA modestly without changing the abundance of the β1-subunit of Na⁺-K⁺-ATPase mRNA (Fig. 3), and 3) increasing the abun-
dance of the α-rENaC mRNA but not the β- or γ-rENaC mRNA levels (Fig. 9).

Steroid hormones and the Na⁺-K⁺ pump. These effects of GC and MC on the pump are somewhat different from those reported for other model systems. In A6 cells, aldosterone produces a greater increase in the α₁-subunit of Na⁺-K⁺-ATPase mRNA, as well as a two- to threefold increase in the β₁-subunit of Na⁺-K⁺-ATPase mRNA produced by steroids. *P < 0.05 compared with all other groups. Values are means ± SE; n = 6 monolayers for each α₁ analysis and 10 monolayers for each β₁ analysis (B).

in a liver cell line while producing a less than twofold effect on the abundance of the α₁-subunit of Na⁺-K⁺-ATPase mRNA (3). It is clear that the effects of steroids on Na⁺-K⁺-pump mRNA levels are cell and tissue specific.

Fig. 8. RNase protection assay of α-, β-, and γ-subunits of rat epithelial Na⁺ channel (rENaC) mRNA demonstrating effect of no steroid or TGF-β₁ (Control), mineralocorticoid (MC) hormone, and TGF-β₁ on IMCD cells. RNA was isolated after 24 h of exposure. Left, length of undigested (Undig) probes; right, length of digested probes.

Fig. 7. Effect of steroid hormone and TGF-β₁ on abundance of α₁- and β₁-subunits of Na⁺-K⁺-ATPase mRNA. A: TGF-β₁ eliminated increase in abundance of α₁-subunit of Na⁺-K⁺-ATPase mRNA produced by steroids. *P < 0.05 compared with all other groups. Values are means ± SE; n = 6 monolayers for each α₁ analysis and 10 monolayers for each β₁ analysis (B).
The present demonstration of an increase in the functional capacity of the Na⁺-K⁺ pump with steroid treatment (Fig. 1) is consistent with our previous results demonstrating an increase in ouabain binding after GC or MC exposure (18). Similar increases in ouabain binding occur in the colon of rabbits fed a low-NaCl diet (40) or in aldosterone-treated A6 cells (2). The results support a general model where steroids increase transcription of one or more of the Na⁺-K⁺-ATPase subunits (albeit modestly), increase pump synthesis (10), and insert more pumps on the basolateral membrane.

Interactions of Na⁺ entry and steroids on the Na⁺-K⁺ pump. The precise molecular mechanisms that lead to the increase in pump activity resulting from steroid stimulation of Na⁺ transport are poorly understood. Considerable evidence suggests that intracellular Na⁺ concentration can influence this process (1, 17, 41). There is also evidence that the increase in transcription of the Na⁺-K⁺-ATPase subunits in A6 cells does not require an increase in protein synthesis or Na⁺ transport (35, 36). Our results generally support the idea that steroids can increase pump capacity without a concomitant increase in intracellular Na⁺ concentration, because GC or MC increases pump capacity in the presence or absence of benzamil (Fig. 4). Although blocking Na⁺ entry with benzamil had a modest effect on the basal pump capacity, the change in pump capacity produced by GC or MC is the same with or without benzamil (Fig. 4). This result is in marked contrast to those reported by Palmer et al. (23) for the rat CCD. They found that amiloride caused a marked reduction in the magnitude of the pump current that was stimulated by aldosterone. The reasons for this difference are not clear, but we suggest two possibilities. First, the use of cultured vs. native cells might influence the nature of the response. Second, the IMCD and CCD respond quite differently to dietary NaCl restriction with respect to ENaC mRNA levels (32). We therefore suspect that fundamental biological differences in cell machinery may be responsible for effecting aldosterone's action on IMCD and CCD cells. The difference in response to amiloride may be one manifestation of these differences.

The effect of steroids on Na⁺-K⁺ pump mRNA levels is sufficiently modest in our system that some limitations are encountered in analyzing the factors that modify the response. Nevertheless, limiting Na⁺ entry with benzamil does not reduce the amount of the α₁ or β₁-subunit of Na⁺-K⁺-ATPase mRNA induced by GC or MC (Fig. 5). Thus these data support the notion that steroids increase the amount of the α₁-subunit of Na⁺-K⁺-ATPase mRNA directly and do not require an increase in Na⁺ entry. However, the scenario may be more complicated. Benzamil actually increases the abundance of the α₁-subunit of Na⁺-K⁺-ATPase mRNA in monolayers treated with MC but not in those treated with GC (Fig. 5). These results, taken together with our previous findings (18), suggest that the action of GC on Na⁺-K⁺ pump activity may be influenced by the magnitude of Na⁺ entry, whereas the action of MC is not.

Interactions of TGF-β₁ and MC on the Na⁺-K⁺ pump. We focused our investigation on the interactions of MC and TGF-β₁, because MC is more physiologically important in regulating IMCD Na⁺ transport than is GC and the effects of TGF-β₁ on GC- and MC-treated IMCD cells are the same (13). In contrast to benzamil, TGF-β₁ completely eliminates the MC stimulation of pump capacity as well as the MC stimulation of Na⁺ transport by intact cells (Fig. 6). In addition, TGF-β₁ also eliminates the increase in the α₁-subunit of Na⁺-K⁺-ATPase mRNA produced by MC without having any effect on the levels in cells not exposed to MC (Fig. 6).

The ratio of the magnitude of Na⁺ transport by intact monolayers to that by the same monolayers after their apical membrane is permeabilized with amphotericin is an estimate of the fraction of the capacity at which the pump operates during normal (steady-state) Na⁺ transport in intact cells. This analysis permits inferences regarding the relative extent of the effects of agents on the Na⁺ channel and Na⁺-K⁺ pump. GC and MC increase the rate of Na⁺ transport in intact cells to a greater degree than they increase the capacity of the pump (Fig. 1). Monolayers treated with MC or GC have a rate of Na⁺ transport that causes the pump to operate at ~50% of its maximal capacity. The pumps in monolayers not treated with steroids operate at ~20% of maximal capacity (Fig. 1). These values are similar to those we reported using IMCD primary cultures from Dahl salt-sensitive and -resistant rats (15). The straightforward interpretation of these results is that steroids increase the entry of Na⁺ across the apical membrane to a greater extent than they increase the capacity of the Na⁺-K⁺ pump to extrude Na⁺. In doing so, steroids cause the Na⁺ concentration in the cell to increase by an estimated 2 mM (15).

TGF-β₁ effects on Na⁺ entry and on the Na⁺-K⁺ pump. TGF-β₁ inhibits the action of MC to increase Na⁺ entry across the apical membrane. This statement is true, because the steady-state Na⁺ transport rate is reduced to values near those of monolayers not treated with steroid. However, in contrast to benzamil, which inhibits only the entry of Na⁺, TGF-β₁ also decreases the effects of MC on the pump (Figs. 6 and 7). The fact that monolayers treated with MC + TGF-β₁ have a pump that operates at the same capacity as monolayers not treated with MC suggests that TGF-β₁ inactivates the responsiveness to steroids. Such a scenario might suggest an action of TGF-β₁ to block the mineralocorticoid and glucocorticoid receptor activation.

However, the interaction of TGF-β₁ and MC seems to be much more complicated than simply blocking steroid receptors. One major action of steroids on IMCD cells is the enhancement of the amount of α₁-ENaC mRNA (Fig. 9) (37). However, TGF-β₁ did not alter this action (Fig. 9). These results are most consistent with the idea that treatment with TGF-β₁ does not alter the MC actions to bind to the mineralocorticoid receptor, activate and translocate to the nucleus (28), and increase the transcription of α₁-ENaC (26). Rather, they suggest that TGF-β₁ induces a posttranscriptional modification of rENaC, such that its full expression is prevented.
There is little precedent for such an action. Perhaps it is because we understand relatively little about the mechanisms of aldosterone’s action that we do not understand how they can be counterregulated. There is one situation, however, where some analogy might be drawn. Aldosterone acts on the CCD to increase the activity of ENaC (22). However, alterations in dietary NaCl that alter circulating aldosterone concentrations and ENaC activity do not alter α-ENaC mRNA levels in renal cortex (32). In contrast, the same maneuvers do produce a change in α-ENaC mRNA levels in the inner medulla (32). These results suggest that increasing the activity of ENaC may be more complex than simply increasing α-ENaC transcription, protein production, and apical membrane delivery. The diversity of tissue and organ responses to dietary NaCl and steroids (19, 32) and the differences in the developmental responses in colon, lung, kidney, and bladder (38, 39) support the notion that regulation of ENaC expression and function is complex.

In conclusion, we have found that TGF-β1 produces antagonism to the action of aldosterone on IMCD cells by complex actions that include preventing the increase in mRNA levels of the α1-subunit of Na+-K+-ATPase and preventing the aldosterone-induced increase in pump capacity. Its antagonism is not manifest at the level of rENaC mRNA, but TGF-β1 does prevent aldosterone from increasing the activity of ENaC. Taken together, these results suggest that aldosterone and TGF-β1 can act in parallel at sites independent of transcription of ENaC to produce counteracting effects on Na+ channel activity.

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