Regulation by Glucocorticoids of Expression and Activity of rBSC1, the Na⁺-K⁺(NH₄⁺)-2Cl⁻ Cotransporter of Medullary Thick Ascending Limb*

Amel Attmane-Elakeb‡§, Valérie Sibella†, Catherine Vernimmen‡, Xavier Belenfant‡, Steven C. Hebert**†‡, and Maurice Bichara‡ ‡‡

To assess whether glucocorticoids regulate rBSC1, the apical Na⁺-K⁺(NH₄⁺)-2Cl⁻ cotransporter of kidney medullary thick ascending limb (MTAL), studies were performed in normal rats, adrenalectomized (ADX) rats, and ADX rats infused with dexamethasone for 6 days. The effects of dexamethasone on rBSC1 were also studied in vitro using isolated rat MTAL segments. Cotransport activity was estimated by intracellular pH measurements; rBSC1 protein was quantified in MTAL crude membranes by immunoblotting analysis, and mRNA was quantified by quantitative reverse transcription-polymerase chain reaction. The abundance of rBSC1 protein and mRNA increased in ADX rats infused with dexamethasone compared with ADX rats (p < 0.04). In addition, application of dexamethasone for 1–3 h to MTALs caused rBSC1 protein and mRNA abundance and cotransport activity to significantly increase in a hyperosmotic medium (450 mosmol/kg of H₂O) containing 0.7 nM arginine vasopressin, which is an in vitro experimental condition that resembles the in vivo MTAL environment. Results obtained in various media and with 8-bromo-cAMP indicated that stimulation of rBSC1 expression by glucocorticoids required interactions between glucocorticoid receptor- and cAMP-dependent factors. Up to 100 nM d-aldosterone had no effect on cotransport activity in vitro. Thus glucocorticoids directly stimulate MTAL rBSC1 expression and activity, which contributes to glucocorticoid-dependent effects on the renal regulation of acid-base balance and urinary concentrating ability.

Na⁺-K⁺(NH₄⁺)-2Cl⁻ cotransport is responsible for the apical step of NaCl and ammonia transport by the thick ascending limb (TAL) of the nephron. NaCl and ammonia absorption without water by the medullary TAL (MTAL) causes transepithelial concentration differences of these solutes, which constitutes the “single effects” responsible for NaCl and ammonia accumulation in the renal medulla. This is critical both to the level of renal medullary hyperosmolality and thus to the urinary concentrating ability of the kidney and to urinary ammonia excretion and thus to the renal regulation of acid-base balance (1, 2). The MTAL apical Na⁺-K⁺(NH₄⁺)-2Cl⁻ cotransporter (BSC1 (bumetanide-sensitive cotransporter) or NKCC2 (Na⁺-K⁺-2Cl⁻ cotransporter)) was recently cloned from rat (3), mouse (4, 5), rabbit (6), and human (7) kidneys. The transporter protein has been localized at the apical membrane of the TAL as well as at the macula densa (8–10). BSC1 was recently shown to be up-regulated by chronic saline loading (9), restriction of water intake and arginine vasopressin (AVP) administration (11), and metabolic acidosis (12) and down-regulated by potassium depletion (13). However, the stimuli and cellular mechanisms of these adaptations of rBSC1 expression were not specified in the latter in vivo studies.

The presence of specific glucocorticoid receptors (GR) in the MTAL has been demonstrated by binding, immunological, and mRNA detection methods (14–17). A number of studies have suggested that in vivo glucocorticoid administration acts on the MTAL, but little is known about the direct effects of glucocorticoids on the functions of this nephron segment. Dexamethasone has been shown to stimulate within a few hours the Na⁺-K⁺-ATPase activity of MTALs incubated in vitro (18, 19). It must be pointed out that glucocorticoids have long been known to contribute to renal urinary concentrating ability through (at least in part) maintenance of medullary hyperosmolality (20) and to the increased urinary excretion of NH₄⁺ in response to metabolic acidosis (21) and that both processes are greatly dependent on Na⁺-K⁺(NH₄⁺)-2Cl⁻ cotransport activity in the MTAL, as pointed out above. These considerations prompted us to design the present study to assess whether glucocorticoids affect rBSC1 expression in the MTAL. To this end we have measured the effects of a 1–3 h in vitro application of glucocorticoids on rBSC1 transport activity and protein and mRNA abundance. We have also determined the abundance of rBSC1 protein and mRNA after in vivo glucocorticoid administration. The results show that rBSC1 expression in the MTAL is up-regulated by glucocorticoids through interactions between GR- and cAMP-dependent pathways.
TABLE I
Composition of experimental solutions. All solutions were adjusted to pH 7.4 with Tris. Solutions were gassed with 100% O₂ and contained 5 mM glucose, 5 mM l-leucine, and 0.1 glitser bovine serum albumin. Solutions A and C with 175 mM NaCl and 70 TiT rators other than NaCl were adjusted to pH 7.4 with Tris. Solutions B and D were used in the fluorometer cuvette to measure NH₄⁺-induced variations in cell pH in the presence of 10 mM barium and 1 μM amiloride (Na⁺-K⁺-(NH₄⁺)-2Cl⁻ cotransport activity).

|       | A           | B           | C           | D           |
|-------|-------------|-------------|-------------|-------------|
| **Urea** | 50          | 50          | 50          | 50          |
| **NaCl** | 125         | 125         | 175         | 175         |
| **KCl**  | 3           | 3           | 3           | 3           |
| **Choline-Cl** | 15         | 15          | 15          | 15          |
| **EGTA** | 10          | 10          | 10          | 10          |
| **K₂HPO₄** | 0.8         | 0.8         | 0.8         | 0.8         |
| **KH₂PO₄** | 0.2         | 0.2         | 0.2         | 0.2         |
| **MgCl₂** | 1           | 1           | 1           | 1           |
| **CaCl₂** | 1           | 1           | 1           | 1           |
| **Hepes** | 10          | 10          | 10          | 10          |

**Experimental Procedures**

**In Vivo Studies—**Male Harlan Sprague-Dawley rats weighing 250–300 g were allowed free access to standard rat chow and drinking solution up to the time of the experiments. Rats were adrenalectomized (ADX) under light ether anesthesia and given 0.9% NaCl in distilled water as drinking solution during 6 days before the experiments. Some ADX rats were administered dexamethasone at 1.2 mg/kg body weight/day, a dose that is known to restore normal glucocorticoid activity, delivered by a micro-osmotic pump (Alza Corporation, Palo Alto, CA) during 6 days (ADX + Dexa); these ADX + Dexa rats also drank 0.9% NaCl in distilled water. Control rats from the same shipments were sham operated and drank normal water or 0.9% NaCl as drinking solution. After anesthesia by sodium pentobarbital, the kidneys were rapidly removed and cut into thin slices along the corticopapillary axis, and under a dissecting microscope the inner stripe of outer medulla of each slice was excised and cut into uniform small pieces, which were used for immunoblotting of membrane proteins and mRNA determinations.

**In Vitro Studies: Suspension of Rat MTAL Tubules—**The method used to isolate MTAL fragments in suspension has been previously described (22). We have established that this suspension was made almost exclusively of MTALs (≥95%), occasional thin limbs, and rare outer medullary collecting tubules, with no isolated cells or proximal tubules (12, 22, 23). MTAL suspensions were not prepared from ADX rats that were given 0.9% NaCl in drinking distilled water for 6 days. Samples destined for rBSC1 protein and mRNA quantification were incubated for 2 h in the presence of dexamethasone or vehicle. Samples destined for measurement of intracellular pH (pHᵢ) to estimate the Na⁺-K⁺-(NH₄⁺)-2Cl⁻ cotransport activity were loaded with 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester and measurements of Na⁺-K⁺-(NH₄⁺)-2Cl⁻ cotransport activity were performed, as described previously (12, 24), after 1–3 h of incubation in the presence of dexamethasone or vehicle. In brief, samples of 2.7–bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester-loaded MTALs, preincubated in solution A or C (Table I), were diluted in the fluorometer cuvette in 2 ml of solution B or D (Table I), and pHᵢ was monitored at 37 °C; then Na⁺-K⁺-(NH₄⁺)-2Cl⁻ cotransport activity was assessed by determining the bumetanide-sensitive component of the cell acidification caused by abrupt exposure to 4 mM NH₄Cl in the presence of 10 mM barium and 1 μM amiloride to block NH₄⁺ carriers other than Na⁺-K⁺-(NH₄⁺)-2Cl⁻ cotransport (12, 24). We have previously demonstrated that the initial rate of NH₄⁺-induced cell acidification (dPᵢ/dt, calculated as described previously (24)) is not significantly affected by changes in the activities of pHᵢ regulatory mechanisms such as Na⁺/H⁺ antport (24). However, we have determined in the present study, by a previously described method (22, 24), that the total Na⁺/H⁺ exchange activity of MTAL fragments in suspension was not affected by 10 mM dexamethasone (data not shown). Thus the initial rate of NH₄⁺-induced cell acidification in the presence of 10 mM barium and 1 μM amiloride will be hereafter referred to as Na⁺-K⁺-(NH₄⁺)-2Cl⁻ cotransport activity.

Crude membranes from the inner stripe of outer medulla or from MTAL suspensions were prepared for immunoblotting studies in the following way. Tissues were homogenized in a medium composed of 125 mM sucrose, 12 mM Trizma (Tris base) (pH 7.4), 0.1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, and 5 μg/ml leupeptin. These homogenates were centrifuged at 100,000 × g for 5 min in a Beckman GS-6KR centrifuge with a GH-3.7 rotor, and the supernatants were further centrifuged at 200,000 × g for 60 min in a Beckman 1-70 Ultracentrifuge. The pellets were suspended in the above medium and stored at ~80 °C until use.

**Electrophoresis and Immunoblotting of Membrane Proteins—**Semi-quantification of membrane protein amounts was performed as described previously (12). In brief, the membranes were solubilized at ambient temperature for 20 min in Laemmli medium containing (final concentrations) 62.5 mM Tris-Cl (pH 6.8), 5% SDS, 100 mM dithiothreitol, 10% glycerol. SDS-polyacrylamide gels were performed with solubilized membranes (15–25 μg of protein) and prestained molecular weight markers (Sigma) on 7.5% polyacrylamide minigels (Mini Protein II, Bio-Rad). Protein was subsequently transferred electrophoretically from the gels to nitrocellulose membranes (Mini Trans Blot Module, Bio-Rad). Equal loading and transfer efficiency were systematically checked by Ponceau red staining of the nitrocellulose membranes. Exposure of the membranes to an anti-rBSC1 polyclonal antibody (8, 12), to an anti-β-actin mouse monoclonal antibody (Sigma-Aldrich Fine Chemicals), and then to the secondary antibodies (peroxidase-linked anti-rabbit Ig and anti-mouse Ig (Bio-Rad)) and quantification of each band were performed as described previously (12). Quantification of β-actin was used as an additional control to check equal loading and transfer efficiency in the nitrocellulose membranes.

**RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction—**Total RNA (RNAᵦᵣ) was extracted from aliquots of the inner stripe of outer medulla or from MTAL suspensions with use of the SV total RNA isolation system kit (Promega). A competitor RNA that differed from the wild rBSC1 mRNA by a 116-base deletion of the latter was obtained, and quantitative reverse transcription-polymerase chain reaction was performed exactly as described previously in detail (12). Amounts of rBSC1 mRNA are expressed in number of RNAᵦᵣ molecules per 100 ng of RNAᵦᵣ. Note that both the rBSC1 protein and mRNA determination methods employed in the present work were designed to detect determinants common to the published rBSC1 isoforms that take place in the rat MTAL (12).

**Materials—**Tag DNA polymerase, Moloney murine leukemia virus reverse transcriptase, and dNTP were obtained from Life Technologies, Inc.; yeast transfer RNA and collagenase Clostridium histolyticum grade II were from Roche Molecular Biochemicals; and 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester was from Molecular Probes (Eugene, OR). Arginine vasopressin, dexamethasone, d-aladosterone, 4-(2-aminoethyl)-benzenesulfonyl fluoride, leupeptin, antipain, aprotinin, 8-bromo-cAMP, and all other chemicals were obtained from Sigma-Chimie S.A.R.L. (LaVerpillière, France).

**Statistics—**Results are expressed as the mean ± S.E. Statistical significance between experimental groups was assessed by Student’s paired or unpaired t test or by 1-way analysis of variance completed by a t test using the within-groups residual variance of the analysis of variance, as appropriate.

**RESULTS**

**In Vivo Studies—**There was no difference in the plasma concentrations of sodium and chloride between three control (145.7 ± 0.3 mS and 98 ± 1, respectively), three ADX (142.7 ± 1.5 and 97 ± 2, respectively), and three ADX + Dexa rats (143.3 ± 0.7 and 96 ± 2, respectively); as expected, the plasma potassium concentration was higher in ADX (5.1 ± 0.5 mS) and ADX + Dexa (5.0 ± 0.2 mS) than in control rats (4.4 ± 0.1 mS). As shown in Table II, the abundance of rBSC1 protein was lower in ADX than in control rats that drank normal water, which was of borderline significance (p < 0.06), or 0.9% NaCl (p < 0.009). However, there was no difference in rBSC1 mRNA abundance. It should be emphasized that, when the normal condition in rats, adrenalectomy is a complex condition in which several factors may have had opposite effects on rBSC1 expression, as discussed below. Thus, in another experimental series, results obtained from 5 ADX rats were compared with those obtained from 5 ADX + Dexa rats. As shown in Fig. 1, dexamethasone administration increased rBSC1 protein abundance in crude membranes of the inner stripe of outer medulla by ~91% (191 ± 23 arbitrary units in...
ADX + Dexa versus 100 ± 10 arbitrary units in ADX; \( p < 0.002 \), whereas there was no change in the abundance of \( \beta \)-actin protein. The dexamethasone-induced increase in rBSC1 protein abundance was accompanied by a \( \approx 43\% \) increase in rBSC1 mRNA abundance (15.7 ± 2.0 amol/100 ng of RNA\(_{tot} \)) in ADX + Dexa versus 11.0 ± 0.7 amol/100 ng of RNA\(_{tot} \); \( p < 0.03 \) (Fig. 2). These results establish that glucocorticoid administration enhances rBSC1 expression in the MTAL.

**In Vitro Studies**—To assess whether glucocorticoids directly stimulate rBSC1 expression in the MTAL in vitro, tubule fragments were incubated in experimental media in the presence of 10 nM dexamethasone or vehicle. In an attempt to recreate the in vivo MTAL environment, we performed in vitro experiments using a moderately hyperosmotic medium (~450 mosmol/kg of H\(_2\)O obtained by adding 50 mM NaCl plus 50 mM urea to isoosmotic medium; solution C in Table I). In addition, this hyperosmotic medium contained 0.7 nM AVP or 0.5 mM 8-bromo-cAMP. Indeed, the MTAL is surrounded in vivo by the hyperosmotic interstitial medium of the inner stripe of outer medulla of the kidney and is submitted to tonic influences by cAMP-generating peptide hormones such as AVP, glucagon, and calcitonin (25). In the hyperosmotic medium containing 0.7 nM AVP, within 2 h of incubation dexamethasone increased the abundance of rBSC1 protein and mRNA. rBSC1 protein abundance increased from 100 ± 4 arbitrary units in control to 137 ± 12 arbitrary units (\( p < 0.02 \); Fig. 3), whereas rBSC1 mRNA abundance increased from 11.0 ± 0.7 amol/100 ng of RNA\(_{tot} \) in control to 12.8 ± 1.9 amol/100 ng of RNA\(_{tot} \) (\( p < 0.04 \); Fig. 3). There was no difference in \( \beta \)-actin protein abundance (100 ± 14 versus 95 ± 30 arbitrary units; \( n = 6 \) for both; NS). The increases in rBSC1 mRNA and protein were accompanied with stimulation of Na\(^+\)-K\(^+\)(NH\(_4\))^+-2Cl\(^-\) cotransport activity. Dexamethasone increased the cotransport activity by \( \approx 25\% \) (\( p < 0.04 \); Fig. 4) within 1–3 h of incubation in the hyperosmotic medium containing 0.7 nM AVP. Stimulation of the cotransport activity by dexamethasone was also observed when 0.5 mM

| Series | \( \beta \)-Actin protein arbitrary units | rBSC1 protein arbitrary units | rBSC1 mRNA amol/100 ng of RNA\(_{tot} \) |
|--------|-----------------------------------------|---------------------------------|---------------------------------|
| I. Sham (\( n = 5 \)) | 100.0 ± 4.7 | 100.0 ± 4.7 | 4.6 ± 0.3 |
| ADX (\( n = 5 \)) | 94.2 ± 6.6 | 84.8 ± 7.5 | 4.0 ± 0.2 |
| II. Sham + NaCl (\( n = 5 \)) | ND | 100.0 ± 2.1 | 10.8 ± 1.1 |
| ADX (\( n = 5 \)) | ND | 82.2 ± 6.4 | 12.8 ± 1.9 |

**Fig. 1.** Upper panel, immunoblot of rBSC1 and \( \beta \)-actin proteins in crude membranes from the inner stripe of outer medulla in five ADX and five ADX + Dexa rats. Lower panel, band densities (arbitrary units) of immunoblots made in duplicate of rBSC1 and \( \beta \)-actin proteins in ADX and ADX + Dexa rats. Bars represent the mean ± S.E. of 10 measurements in each group. Statistical comparisons were made by unpaired Student’s t tests.

**Fig. 2.** Determination of rBSC1 mRNA abundance (amol/100 ng of RNA\(_{tot} \)) in the inner stripe of outer medulla of five ADX and five ADX + Dexa rats. Statistical comparison was made by an unpaired Student’s t test.

**Fig. 3.** Effects of 10 nM Dexa on the abundance of rBSC1 protein (arbitrary units) and mRNA (amol/100 ng of RNA\(_{tot} \)) in MTALs after 2 h of incubation in hyperosmotic medium containing 0.7 nM AVP. The upper panel shows a representative immunoblot of rBSC1 protein. The bars in the lower left panel represent the mean ± S.E. of six determinations.
8-bromo-cAMP was added in place of AVP in the hyperosmotic medium (the dpH/dt was −0.86 ± 0.05 versus −0.66 ± 0.03 pH unit/min in controls; n = 18 and 20, respectively; p < 0.002). By contrast, dexamethasone had no effect on \( \text{NH}_4^- \cdot \text{K}^- \cdot (\text{NH}_4^+) \cdot 2\text{Cl}^- \) cotransport activity (−1.20 ± 0.04 versus −1.17 ± 0.08 pH unit/min in controls; n = 6 and 7, respectively; NS). Furthermore, dexamethasone-induced stimulation of cotransport activity in the hyperosmotic medium containing AVP was abolished by 20 \( \mu \text{M} \) actinomycin D or 20 \( \mu \text{M} \) cycloheximide (Fig. 4), which are inhibitors of transcription and protein synthesis, respectively.

To further study the interactions between dexamethasone and AVP or the experimental medium, dexamethasone was applied for 2 h to MTALs incubated in an isoosmotic medium (−300 mosmol/kg of \( \text{H}_2\text{O} \); solution A in Table I) containing 0.7 nm AVP or in AVP-free hyperosmotic and isoosmotic media. In the isoosmotic medium containing 0.7 nm AVP, dexamethasone again strongly increased both rBSC1 protein abundance from 100 ± 9 arbitrary units in control to 212 ± 18 arbitrary units (p < 0.0004; Fig. 5) and mRNA abundance from 7.3 ± 0.4 amol/100 ng of RNA\(_{\text{tot}}\) in control to 21.0 ± 3.4 amol/100 ng of RNA\(_{\text{tot}}\) (p < 0.04; Fig. 5). There was no difference in \( \beta \)-actin protein abundance (100 ± 3 arbitrary units in controls versus 107 ± 6 arbitrary units; n = 6 for both; NS). By contrast, in the AVP-free hyperosmotic medium, dexamethasone decreased rBSC1 protein abundance from 100 ± 1 arbitrary units in control to 62 ± 5 arbitrary units (p < 0.0001; Fig. 6) but did not affect rBSC1 mRNA abundance (4.5 ± 1.0 amol/100 ng RNA\(_{\text{tot}}\) in control versus 4.5 ± 1.1 amol/100 ng RNA\(_{\text{tot}}\); NS; Fig. 6). There was no difference in \( \beta \)-actin protein abundance (100 ± 4 arbitrary units in controls versus 96 ± 7 arbitrary units; n = 6 for both; NS). Finally, in the AVP-free isoosmotic medium, dexamethasone decreased the abundance of rBSC1 protein and mRNA in MTAL suspensions within 2 h. rBSC1 protein abundance decreased from 100 ± 4 arbitrary units in control to 74 ± 8 arbitrary units (p < 0.02; Fig. 7), whereas rBSC1 mRNA abundance decreased from 2.8 ± 0.3 amol/100 ng of RNA\(_{\text{tot}}\) in control to 2.1 ± 0.4 amol/100 ng of RNA\(_{\text{tot}}\), which was of borderline significance (p < 0.06; Fig. 7). There was no difference in \( \beta \)-actin protein abundance (100 ± 8 versus 80 ± 24 arbitrary units; n = 4 for both; NS). The decreases in rBSC1 mRNA and protein abundance were accompanied by inhibition of Na\(^-\)K\(^+\) transport activity. Indeed, 10 nm dexamethasone decreased the cotransport activity by −27% within
Glucocorticoids Regulate MTAL rBSC1 Expression

This study is the first, to our knowledge, in which possible effects of glucocorticoids on rBSC1 expression in the MTAL were assessed both in vivo and in vitro. Comparing the level of rBSC1 expression in ADX rats to that in normal rats to assess the effects of glucocorticoid deficiency can hardly be achieved satisfactorily because adrenaleceomy is a complex condition with respect to rBSC1 expression. For example, adrenaleceomy is associated with increased circulating AVP concentrations probably because of impaired cardiac function (26), and AVP administration and cardiac insufficiency, even with normal AVP levels, both have been shown to strongly stimulate rBSC1 expression in the MTAL (11, 27). In addition, NaCl administration, which was used in ADX rats to minimize urinary NaCl losses, has also been shown to stimulate rBSC1 expression (9). With these issues in mind, we observed that the abundance of rBSC1 protein in ADX rats tended to be lower as compared with rats given normal water (p < 0.06) and was significantly lower as compared with rats given 0.9% NaCl as drinking solution, like ADX rats (p < 0.009); however, there was no significant difference in rBSC1 mRNA abundance in both experimental series. On the other hand, supplementing ADX rats with dexamethasone appears to be a better means of assessing the effects of glucocorticoids on rBSC1. Glucocorticoid administration to ADX rats strongly stimulated rBSC1 mRNA and protein expression in the MTAL as compared with ADX rats. Thus these results establish that glucocorticoids enhance rBSC1 expression when administered in vivo. Furthermore, when dexamethasone was directly applied to MTALs in vitro, rBSC1 mRNA and protein expression and cotransport activity were stimulated in a hyperosmotic medium containing AVP, an experimental condition that resembles the natural MTAL environment. Strong stimulation of rBSC1 expression by dexamethasone was observed in an AVP-containing isoosmotic medium as well, and a glucocorticoid-induced increase in Na+-K+-2Cl⁻ cotransport activity also occurred when 8-bromo-cAMP was used in place of AVP in a hyperosmotic medium. The stimulating effects of dexamethasone were not seen in AVP- and cAMP-free media. Thus it is clear that stimulation of rBSC1 expression by dexamethasone required interactions with cAMP-dependent factors. Because several peptide hormones such as AVP, calcitonin, and glucagon stimulate the MTAL adenylyl cyclase, it is very likely that MTAL cells in vivo are chronically subjected to the influences of cAMP-generating peptide hormones (25). The in vitro effects of dexamethasone on cotransport activity were abolished by actinomycin D or cycloheximide and were not observed with d-aldehyde. This indicates that GR activation and attendant effects on gene transcription and translation were responsible for the changes in rBSC1 expression and activity. The present finding that glucocorticoids physiologically stimulate rBSC1 expression and activity would be consistent with the observations that dexamethasone also stimulates Na⁺-K⁺-2Cl⁻ cotransport activity (18, 19). Both of these effects would provide the mechanism for glucocorticoid-dependent stimulation of NaCl and NH₄⁺ transport by the TAL.

The intracellular mechanisms by which glucocorticoids exerted their effects in the MTAL were not investigated in the present study. As mentioned above, GR activation in vitro appears to be able to stimulate as well as to inhibit rBSC1 expression and activity depending on the presence or absence of cAMP. The results obtained in the various experimental media suggest that several interactions between GR activation and osmolality- and cAMP-dependent factors take place in the MTAL to physiologically enhance rBSC1 expression. Thus a number of intracellular events, such as altered rBSC1 gene

FIG. 8. Effects of 10 nm Daxa on Na⁺-K⁺-(NH₄)₂-2Cl⁻ cotransport activity in MTALs after 1–3 h of incubation in AVP-free isoosmotic medium. The bars represent the mean ± S.E. of 9–11 determinations. C, control; Actino., 20 µM actinomycin D; Cyclo., 20 µM cycloheximide.

FIG. 9. Effects of 10 and 100 nm d-aldehyde on Na⁺-K⁺-(NH₄)₂-2Cl⁻ cotransport activity in MTALs after 1–3 h of incubation. The bars represent the mean ± S.E. of six determinations. C, control; Aldo., d-aldehyde.

1–3 h of incubation (p < 0.001; Fig. 8). Dexamethasone had no effect on NH₄⁺-induced dPH/dt in the presence of 0.1 mM bumetamide (-1.05 ± 0.06 versus -1.09 ± 0.05 pH unit/min in controls; n = 12 for both; NS). Furthermore, dexamethasone-induced inhibition of cotransport activity in the isoosmotic medium was abolished by 20 µM actinomycin D or 20 µM cycloheximide (Fig. 8). Thus the stimulating effects of dexamethasone required the presence of AVP in both hyperosmotic and isoosmotic media.

To validate the changes in dPH/dt in the presence of 10 mM barium plus 1 µM amiloride described above as reflecting the effects of glucocorticoids on the Na⁺-K⁺-(NH₄)₂-2Cl⁻ cotransport activity, the following control experiments were performed. Dexamethasone had no effect on the cell-buffering capacity (76 ± 6 versus 80 ± 4 mmol of H⁺/pH unit/liter; NS) or on the cell volume (0.39 ± 0.01 versus 0.39 ± 0.01 nl/mm of tubule length; NS); cell buffering capacity and cell volume were estimated exactly as described previously (24). We have also checked that, after up to 3 h of incubation, Na⁺-K⁺-(NH₄)₂-2Cl⁻ cotransport activity was inhibited by the hyperosmotic medium (from -0.59 ± 0.07 pH unit/min in the isoosmotic medium to -0.43 ± 0.04 pH unit/min; p < 0.05) and was stimulated by 0.5 mM 8-bromo-cAMP in the isoosmotic medium (from -0.62 ± 0.09 pH unit/min in controls to -0.95 ± 0.02 pH unit/min; p < 0.01). Finally, 10 and 100 nm d-aldehyde had no effect on the Na⁺-K⁺-(NH₄)₂-2Cl⁻ cotransport activity (Fig. 9).
transcription, mRNA decay, translation efficiency, and membrane trafficking of rBSC1 protein, may have combined to explain our results. Regulation of accessory protein expression may also have occurred. It is well known that the GR can interact with several other proteins through protein-protein interactions or with transcription factors at the level of the promoters of regulated genes, which may be controlled by cAMP. In particular, glucocorticoid-induced GR activation was shown, through interactions with the transcription factor AP1 at the level of a composite glucocorticoid response element named pGRE, to activate or inhibit transcription depending on the c-Jun-c-Jun or c-Jun-c-Fos composition of AP1 (28). Further work is needed to address these issues in MTAL cells.

Thus the present results establish that in vitro glucocorticoid administration as well as in vitro glucocorticoid application to freshly harvested MTALs in media containing AVP stimulates rBSC1 expression in rat MTAL, as manifested by an increase in freshly harvested MTALs in media containing AVP stimulates administration as well as urinary NH₄⁺ excretion in response to acid loading (30–32). This acidosis (12) and that adrenal glucocorticoid production is emphasized that we have recently established that rBSC1 expression in the MTAL is enhanced during chronic metabolic acidosis (12) and that adrenal glucocorticoid production is known to increase in response to acid loading (30–32). This suggests that glucocorticoids act in both the proximal tubule and MTAL to increase urinary NH₄⁺ excretion in response to metabolic acidosis. In addition, we have also shown that in vitro incubation of MTALs in an acid medium enhances rBSC1 mRNA and protein abundance and cotransport activity (12). Thus the direct effects of an acid pH and of glucocorticoids would add to fully explain the stimulation of rBSC1 expression in the MTAL by metabolic acidosis (Ref. 12 and present study). Thus the direct effects of an acid pH and of glucocorticoids would add to fully explain the stimulation of rBSC1 expression in the MTAL by metabolic acidosis (Ref. 12 and present study).

Further work is needed to test this hypothesis. Otherwise, it is worth noting that, if glucocorticoids stimulate rBSC1 expression in the cortical TAL also, sodium chloride absorption without water should be enhanced along the entire TAL, which would contribute to explaining the permissive role of glucocorticoids in the renal elimination of a water load (33). The effects of glucocorticoids on rBSC1 described in the present study may thus explain, at least in part, the well known inability of the kidney to maximally concentrate or dilute the urine during adrenal insufficiency.

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