Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 510 (2005) 181–186

www.elsevier.com/locate/ejphar

Aldosterone stimulates active Na^+ transport in rabbit urinary bladder by both genomic and non-genomic processes

Timothy J. Burton, Dermot M.F. Cooper, Bryony Dunning-Davies, Dina Mansour, Nanako Masada, Douglas R. Ferguson*

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1PD, UK

Received 3 January 2005; accepted 13 January 2005

Abstract

The ability of aldosterone to stimulate Na^+ transport in a range of epithelial tissues has been known for many years. Early work suggested that aldosterone had a delayed action operating by transcriptional up-regulation of proteins such as the epithelial Na^+ channel. However more recent data has suggested that the hormone has a short-term non-genomic action. In this paper we investigate short and long-term actions of aldosterone on Na^+ transport in the rabbit urinary bladder. We have shown that aldosterone stimulates epithelial Na^+ channel activity, as measured by the amiloride-sensitive short-circuit current over a 3.75 h period and that this action is potentiated by cAMP. Using reverse transcriptase-polymerase chain reaction we have shown that aldosterone and forskolin in combination up-regulate mRNA synthesis for the β- and γ-subunits of the epithelial Na^+ channel. Using Western blotting we have shown in the case of the β-subunit that a corresponding increase in channel protein occurs. We have also demonstrated that aldosterone in the presence of inhibitors of phosphodiesterase can stimulate the short-circuit current across rabbit bladder epithelium over a 20 min period. An explanation for the synergistic interaction between aldosterone and cAMP is provided. We have shown that aldosterone can increase cAMP levels within urothelial cells over a 4 min period. We propose that this represents a non-genomic action of the steroid hormone.

Keywords: Urinary bladder; Epithelial Na^+ channel; Aldosterone

1. Introduction

Aldosterone has been known to increase Na^+ transport in amphibian urinary bladders since 1961 (Crabbe). This effect was demonstrated 24 h after injection of the hormone into the toad, *Bufo marinus*. Subsequent experiments with actinomycin D and puromycin (Edelman et al., 1963; Crabbe and de Weer, 1964) showed that the increase in Na^+ transport in response to the hormone was blocked if protein synthesis was inhibited. The ability of aldosterone to stimulate Na^+ transport in mammalian bladders was demonstrated by Wickham (1964) and Lewis and Wills (1983). The results of these experiments were in accord with the view that steroid hormones act physiologically as transcriptional regulators, and thereby induce the synthesis of new proteins. Although these early experiments identified transport of Na^+ through the apical membranes of urothelial cells as the rate limiting step for transmembrane sodium flux, the three subunits comprising the epithelial Na^+ channel (ENaC) which carry the Na^+ ions across the membrane were not identified until 1994 (Canessa et al.) in rat colon. These authors also confirmed the sensitivity of epithelial Na^+ channels to block by amiloride.

More recently Sheader et al. (2002) demonstrated that aldosterone increases cAMP levels in renal cortical collecting ducts over short time periods, thereby increasing Na^+ transport and demonstrating a non-genomic action of the hormone. The experiments reported in this paper investigate the actions of aldosterone on the rabbit bladder and reveal that these are both long and short term, and have the effect of enhancing active transepithelial Na^+ transport.

* Corresponding author. Tel.: +44 1223 240651; fax: +44 1223 334040.
E-mail address: drfig33@doctors.org.uk (D.R. Ferguson).

0014-2999/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2005.01.017

2. Methods

2.1. Animals and reagents

The animals used were small Dutch rabbits (~1 kg) of either gender. They were cared for and killed according to Home Office guidelines. Reagents unless otherwise indicated were obtained from Sigma, Poole, UK.

2.2. Ussing chamber experiments

Urinary bladders were dissected free, washed in Krebs' solution and halved vertically. Each hemi-bladder was mounted separately between the two halves of an Ussing chamber, and clamped in position to act as a membrane, the electrical properties of which could be measured. The area of tissue exposed to the medium on each side was 0.2 cm^2. A DVC-1000 Dual Voltage Clamp (World Precision Instruments, FL, USA) was used to measure transepithelial voltage, and to clamp this potential difference to zero giving the short-circuit current. This has been demonstrated to correspond to the net transmembrane sodium flux in rabbit bladders (Lewis and Diamond, 1976). Transepithelial electrical potential difference was measured using KCl-agar bridges connected via Russell calomel electrodes (Russell, Auchtermuchty, Scotland) and the current passed via silver–silver chloride bridges. The bladders were oxygenated with 95% O_2 and 5% CO_2 and maintained at 37 °C. The composition of the Krebs' solution was (mM) NaCl 124, KCl 5, $MgCl_2$ 1.3, $NaHCO_3$ 26, $CaCl_2$ 1.1, KH_2PO_4 1.4 and glucose 10.

2.3. RNA extraction and reverse transcriptase-polymerase chain reaction

RNA was isolated from urothelial cells firstly by homogenising them in Tripure isolation reagent (Boehringer, Mannheim, Germany). This solution was extracted with chloroform, then propanol. Samples were centrifuged at 16,000×*g* for 15 min at 4 °C and the pellet taken up in ethanol and re-centrifuged. The final pellet was taken up in 20 ml sterile water and dissolved at 60 °C for 5 min. Samples were treated with a DNA-free kit (Ambion, TX, USA) to remove remaining DNA, and the RNA quantified by absorbance at 260 nM. Purity and integrity of the resulting RNA was assessed on agarose gel electrophoresis which demonstrated 28S and 18S rRNA as well defined bands.

Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using kits from HTBiotechnology Ltd. (Cambridge, UK) using the primers listed in Table 1.

Magnesium concentrations and cycling conditions were optimised for each pair of primers. These are outlined as follows. The optimal magnesium concentration was 2.0 mM for the β-subunit of the epithelial Na^+ channel, 2.5 mM for γ-subunit and β-actin, and 3.0 mM for α-subunit. Optimal parameters for the three stage cycling reaction were as follows: 30 s at 94 °C (denaturation); 30 s at 58 °C (54 °C in the case of γ-subunit) (primer annealing); and 30 s (1 min in the case of β-subunit) at 72 °C (primer extension). The number of denaturation-annealing-extension cycles required varied for each primer pair.

Table 1
Primer sequences for RT-PCR amplification of β-actin and rabbit epithelial Na^+ channel (rbENaC) subunits

Gene	Primer	Sequence	Product length (bp)
α-rbENaC	Forward	5′-tgg cga gga aag act gg-3′	417
	Reverse	5′-tca tcc tgt ccg tgc ac-3′	
β-rbENaC	Forward	5′-ctg aag ctg atc ctg gac-3′	753
	Reverse	5′-caa tga tga tct cgg caa ac-3′	
γ-rbENaC	Forward	5′-cga gat gct tct gtc cca at-3′	234
	Reverse	5′-cag gtc gtc gtc tat ctc-3′	
β-actin	Forward	5′-gct acg agc tgc ctg acg g-3′	328
	Reverse	5′-gag gcc agg atg gag cc-3′	

The expected size of product amplified by the four pairs of primers is given, based on the published mRNA sequences that code for these proteins. Accession numbers for the mRNA sequences of α-, β- and γ-subunits of the rabbit epithelial Na^+ channel are, respectively, AJ132108, AJ132109 and AJ132110.

PCR products were visualised under UV light and quantified using a UVI band software programme (UVItec Ltd., Cambridge, U.K). Restriction enzyme treatment of PCR products of all three subunits yielded the expected products.

2.4. Western blotting

Western blotting was used to investigate the effects of aldosterone on the expression of epithelial Na^+ channel subunit proteins. Rabbit urinary bladder epithelium (~100 mg of tissue) was suspended in 5 ml of hypotonic cell lysis buffer containing 100 mM Tris–HCl, 1 mM EDTA, 1 mM benzamidine hydrochloride and 1 mM AEBSF (4-(2-aminoethyl)benzenesulphonylfluoride). The tissue was homogenised to a cloudy suspension using a Polytron® PCU-2. The protein concentration of each sample was determined using a BCA protein assay (Pierce, IL, USA) containing bicinchoninic acid.

Polyacrylamide gels were cast 1.0 mm in thickness. The composition of the lower separating gel was10% (w/v) acrylamide, 375 mM Tris–HCl (pH=8.8), 0.1% (w/v) sodium dodecyl sulphate (SDS), 0.05% (w/v) ammonium persulphate and 0.05% (v/v) *N*,*N*,*N*′,*N*′-tetra-methyl-ethylenediamine (TEMED). The composition of the upper stacking gel was 4.2% (w/v) acrylamide, 125 mM Tris–HCl (pH=6.8), 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulphate and 0.08% (v/v) TEMED.

Protein samples were mixed with sample buffer in the volumetric ratio 4 protein: 1 buffer. Sample buffer had the composition 250 mM Tris base, 16% (w/v) SDS, 40% (v/v) glycerol, 20% (v/v) β-mercaptoethanol and 0.04 mg/ml bromophenol blue (pH=8.7). The volume of each sample loaded was adjusted on the basis of the protein concentration to achieve approximately equal amounts of protein per well.

A SeeBlue® protein ladder (Invitrogen) was run adjacent to the samples for molecular weight determination.

Electrophoresis of samples was performed in tank buffer cooled on ice. The composition of tank buffer was 25 mM Tris base, 190 mM glycine and 0.05% (w/v) SDS (pH=8.3). A voltage of 40 V was applied for 30 min and then increased to 120 V for 2.5 h.

The separating gel was immersed in transfer buffer containing 48 mM Tris base, 39 mM glycine, 0.038% (w/v) SDS and 20% (v/v) methanol. The gel was positioned in contact with a nitrocellulose membrane and cushioned between two graphite electrode plates by filter papers soaked in transfer buffer. A current of 38 mA was passed between the electrodes for 1.5 h to transfer the negatively charged protein onto the nitrocellulose membrane.

The G-protein subunit $G_{\alpha,q/11}$ was used as an internal control to assess the relative amounts of plasma membrane protein between samples. $G_{\alpha,q/11}$ was detected using a rabbit polyconal antibody (Santa Cruz Biotechnology) at a 1 in 500 dilution (working concentration of 0.4 μg/ml). A variety of antibodies were screened for their ability to recognise rabbit epithelial Na^+ channel subunits. Two rabbit polyclonal antibodies (gift from C. Fuller and D. Benos, University of Alabama at Birmingham, USA) raised against peptide sequences from the α-subunit and β-subunit of human epithelial Na^+ channel were also screened.

A goat anti-rabbit secondary antibody (Bio-Rad Laboratories, CA, USA) was used to visualise binding of the rabbit polyclonal antibodies. The secondary antibody was conjugated to horseradish peroxidase (HRP) and used at a 1 in 2000 dilution. All antibodies were diluted in filtered milk-TBST (Tris buffered saline with Tween) solution.

Nitrocellulose membranes were incubated with primary antibody for 1.5 h at room temperature. Primary antibody was applied to either the whole nitrocellulose membrane or to distinct areas of the membrane using a Mini-PROTEAN II multiscreen apparatus (Bio-Rad). After incubation, non-specific binding of primary antibody was removed by three 10-min washes in TBST. Secondary antibody was applied for 30 min at room temperature and the three 10-min washes repeated.

ECL™ Western blotting detection reagent (Amersham Pharmacia Biotech) was applied to the nitrocellulose membrane (0.125 ml/cm^2) for 1 min and removed. This detection reagent contains a cyclic diacylhydrazide that is converted to a luminescent compound by HRP. Luminescence was detected by placing the nitrocellulose membrane wrapped in Saran™ wrap adjacent to blue-light sensitive autoradiography film (Hyperfilm ECL). Films were exposed for various time periods (1–15 min) and developed.

2.5. Measurement of cAMP accumulation

Cyclic AMP accumulation in intact cells was measured as described previously (Evans et al., 1984) with some modifications (Fagan et al., 1996). Urothelial cells scraped from rabbit bladders were incubated in F-10 medium (90 min at 37 °C) with [2-^{3}H]-adenine (20 μCi/dish) to label the ATP pool. The cells were then washed once with phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4, pH 7.4) containing EDTA (0.03%), and resuspended in a nominally calcium free Krebs' buffer containing 120 mM NaCl, 4.75 mM KCl, 1.44 mM $MgSO_4$, 11 mM glucose, 25 mM HEPES and 0.1% bovine serum albumin (fraction V) adjusted to pH 7.4 with 2 M Tris base. The cell suspension from one 100-mm dish was aliquoted into 12 tubes for cAMP determination. The use of calcium free Krebs' buffer in experiments denotes the addition of 0.1 mM EGTA to the nominally calcium free Krebs' buffer. All experiments were carried out at 30 °C in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (500 μM), which was preincubated with the cells for 10 min prior to a 4-min assay. Assays were terminated by addition of 10% (w/v final) trichloroacetic acid. Markers were added to monitor the recovery of cAMP and ATP. After pelleting the [^{3}H]-ATP and [^{3}H]-cAMP content of the supernatant were quantified as described previously (Fagan et al., 1996). Accumulation of cAMP is expressed as the conversion of [^{3}H]-ATP into [^{3}H]-cAMP.

2.6. Statistics

Statistical analysis of differences between means was undertaken using Student's *t*-test, with $P<0.05$ as significant.

3. Results

3.1. Genomic actions of aldosterone on the urinary bladder

The addition of aldosterone (5 μM) to the basolateral surface of the rabbit bladder mounted in an Ussing chamber produced a modest but statistically significant increase in

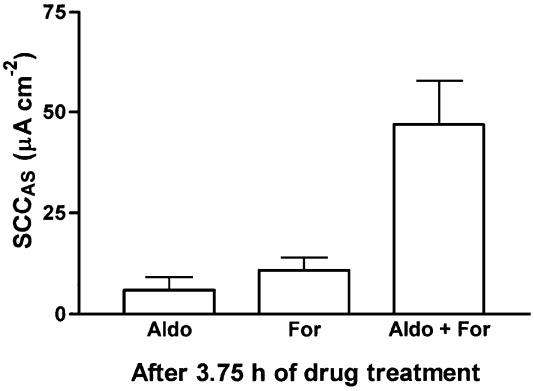

Fig. 1. Increase in amiloride-sensitive short-circuit current (SCC_{AS}) across rabbit urinary bladder 3.75 h after addition of aldosterone (5 μM), forskolin (3 μM), or a combination of the two. The increase produced by aldosterone and forskolin together is significantly greater than either drug alone ($P<0.05$). Values are given as means±S.E.M. ($n=5$).

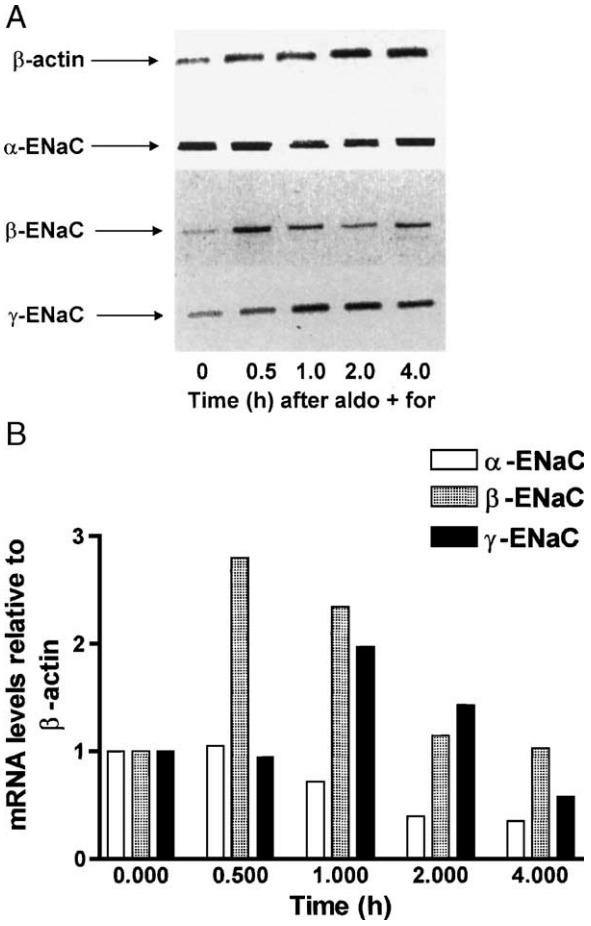

Fig. 2. Effect of combined aldosterone and forskolin treatment on epithelial Na^+ channel (ENaC) mRNA levels over a 4-h time period. RNA was extracted from rabbit bladder urothelium immediately before and 0.5, 1.0, 2.0 and 4.0 h after incubation with a combination of 5 μM aldosterone and 3 μM forskolin. After reverse transcription of the RNA into cDNA, PCR reactions were performed to amplify transcripts of α-ENaC (29 cycles), β-ENaC (31 cycles), γ-ENaC (38 cycles) and β-actin (20 cycles). The PCR reaction products are shown in (A). The intensity of each band was quantified and expressed as a ratio relative to the β-actin band in the same sample (B). For each ENaC subunit, the ratio ENaC/β-actin was normalised to one for tissue prior to aldosterone and forskolin treatment. Intensity values have been expressed relative to the intensity at time t=0, i.e. immediately prior to drug treatment. Values are given as the mean of two PCR reactions using tissue from one animal.

short-circuit current over a 3.75 h period (Fig. 1). This increase continues for at least 6 h (data not shown). Forskolin (3 μM) had a similar action. However the addition of aldosterone and forskolin together produced a massive potentiation of the short-circuit current (Burton et al., 2002).

The effects of mixtures of aldosterone and forskolin together on urothelial cell levels of mRNA coding for the three epithelial Na^+ channel subunits were next examined. RNA was extracted from urothelial cells (as described in Methods), immediately before and 0.5, 1, 2 and 4 h after incubation with a combination of aldosterone (5 μM) and forskolin (3 μM). The drugs were maintained in the organ bath throughout these experimental periods. After reverse transcription of the RNA into cDNA the products were separated by electrophoresis on agarose gels. β-Actin was used as a marker gene. Fig. 2A shows the results of such a gel separation. It reveals that the mRNA coding for the β- and γ-subunits increases after the exposure of the cells to the mixture of aldosterone and forskolin, but that the RNA coding for the α-subunit is unchanged. This result is summarised in Fig. 2B where the intensity of the individual bands is expressed as a ratio relative to the β-actin band in the same sample.

Protein expression of epithelial Na^+ channel subunits is difficult to estimate in the rabbit as most commercially available antibodies are raised in rabbits. Fortunately the β-subunit of the rabbit epithelial Na^+ channel is sufficiently similar in sequence to that of the human to cross-react with antibodies raised against the human subunits. The results of Western blotting are shown in Fig. 3. Antibody to the $G_{\alpha,q11}$ protein was used as a comparison. It has been shown that expression of this G-protein subunit is not altered by aldosterone (Kurrasch et al., 2004). Antibody to β-epithelial Na^+ channel subunits was used at dilutions of 1:250, 1:750 and 1:1500 as indicated. Multiple proteins were visualised in each case, with major bands ~55–60 kDa, and smaller bands at 30, 75 and 95 kDa. After treatment of the tissue with aldosterone and forskolin the antibody was able to detect protein at 1:1500 dilution, whereas no such bands were detected in control tissue. We have been unable to obtain antibodies for use in Western blotting which recognise the α- and γ-subunits of rabbit epithelial Na^+ channels.

3.2. Non-genomic actions of aldosterone on Na^+ transport in bladder

Two series of experiments were undertaken to investigate the short-term actions of aldosterone on the urinary

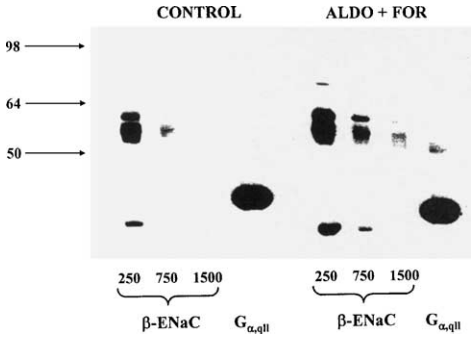

Fig. 3. Effect of combined aldosterone and forskolin treatment on β-ENaC protein expression. The urothelium from a rabbit urinary bladder was divided equally and one-half incubated at 37 °C with 5 μM aldosterone and 3 μM forskolin for 4 h. The remaining tissue was employed as a solvent control. A crude membrane fraction was obtained from both hemibladders, separated by SDS-PAGE and transferred onto a nitrocellulose membrane. Drug-treated (ALDO+FOR) and control samples were probed with an antibody specific for the G-protein subunit $G_{\alpha,q11}$ (1:500 dilution), and with three dilutions (1:250, 1:750 and 1:1500) of an antibody raised against a peptide sequence from the β-subunit of human ENaC. Molecular weight markers (in kDa) are provided to the left. The image shown is representative of 3 identical experiments.

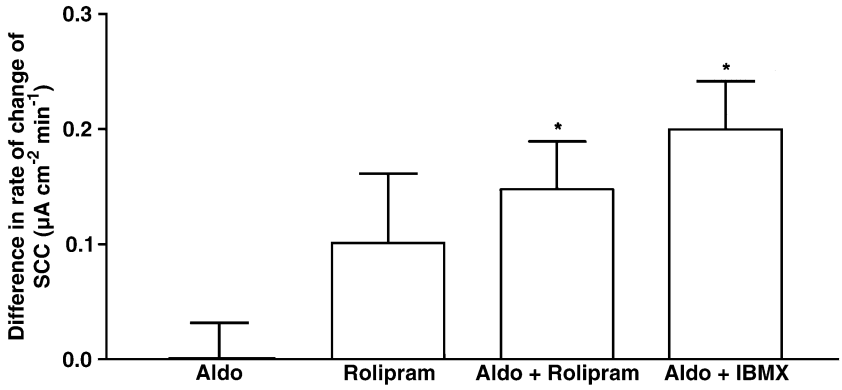

Fig. 4. Rapid actions of aldosterone on Na^+ transport by rabbit bladders. Bladder epithelia were mounted in Ussing chambers and the short-circuit current was measured. The rate of change of short-circuit current with time was measured before and 20 min after the addition of a drug. The difference in rate of change of current after drug addition is shown. Values are given for aldosterone (5 μM), rolipram (1 μM, a phosphodiesterase type IV inhibitor) and aldosterone in combination with rolipram and 3-isobutyl-1-methylxanthine (IBMX), 100 μM, a non-specific phosphodiesterase inhibitor. Values are given as means±S.E.M. (n=5). * Denotes P<0.05 (paired t-test).

bladder. In the first series Ussing chamber experiments were used to measure the short-circuit current changes in response to added aldosterone with or without inhibitors of phosphodiesterase. The results are expressed as the difference in the rate of change of electrical current with time (Fig. 4). This was calculated by measuring the gradient of the current–time trace immediately before and then 20 min after the addition of a drug. The tabulated values show the subtracted difference in the two gradients. A positive value indicates a stimulatory effect on the short-circuit current. Aldosterone alone and rolipram (an inhibitor of type 4 phosphodiesterase) alone have no significant stimulatory action on short-circuit current over a 20-min period. However the combination of aldosterone and a phosphodiesterase inhibitor (either rolipram or 3-isobutyl-1-methylxanthine) produces a significant activation of current within 20 min.

The second series of experiments were undertaken to measure urothelial cell levels of cAMP. The results show that cAMP concentrations are significantly elevated by aldosterone within 4 min of addition of the steroid (Fig. 5).

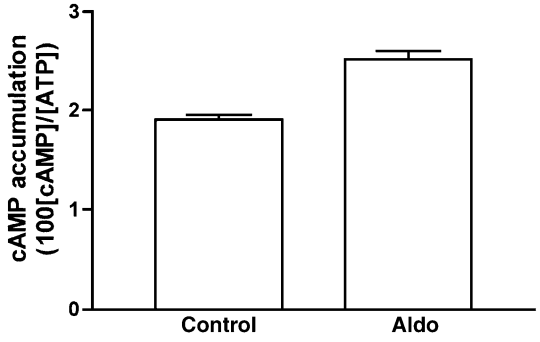

Fig. 5. Cellular levels of cAMP 4 min after addition of aldosterone. The results are expressed as the percentage conversion of labelled ATP to cAMP per mg of protein over a 3-min period. cAMP levels in control urothelial cells and in those incubated with 10 nM aldosterone are shown. The difference is significant P<0.05.

4. Discussion

Although the physiological role of the urinary bladder is simple, namely to collect and store urine and to expel it at appropriate intervals, the active transport of Na^+ by this organ is complex and a very dynamic process. Regulation of the rate of such Na^+ transport is required not for bodily Na^+ homeostasis, but for the maintenance of the appropriate sensitivity of the urothelial hydrostatic pressure sensing mechanism described by Ferguson et al. (1997), and further elaborated by Burton et al. (2002). The work described in these papers shows that the magnitude of urothelial active transport of Na^+ depends on the hydrostatic pressure exerted on the membrane, the urinary Na^+ concentration and the concentrations of aldosterone to which the tissue is exposed. The experimental results presented in this paper indicate that the effects of aldosterone to stimulate Na^+ transport are seen within minutes but also last for several hours, and are therefore likely to be both non-genomic and genomic.

The long-term genomic actions of aldosterone result in increases in mRNA coding for only the β- and γ-subunits of the epithelial Na^+ channel, but not the α-subunit. This finding is similar to that of Renard et al. (1995) in which treatment with dexamethasone increased only β- and γ-subunits in colon. Ecelbarger et al. (2000) demonstrated that vasopressin induced synthesis of β- and γ-subunits in renal cortical collecting ducts, whereas the actions on the α-subunit were much more modest. It is reasonable to conclude from this that in several different tissues epithelial Na^+ channel numbers at the apical cell membranes are controlled through two out of the three subunits needed to produce functional channels, with the third subunit being constitutively expressed.

It was originally envisaged that in epithelial Na^+ channel containing tissues vasopressin was a short-acting, but rapidly effective agent, whereas aldosterone was responsible for a slower and more prolonged elevation in Na^+ transport (Bentley, 1966). In fact both hormones have both long and

short-term actions, both stimulate production of cAMP, and they appear from the results of Ecelbarger et al. (2000) to potentiate each other's actions. However in urinary bladder, although forskolin potentiates the action of aldosterone, we have been unable to demonstrate a natriferic action of vasopressin alone (Burton et al., 2002).

Sheader et al. (2002) showed in the renal inner medullary collecting duct that aldosterone induced production of cAMP within 4 min, and importantly that both vasopressin and aldosterone induced cAMP accumulation in an additive fashion through different receptors. The present results indicate the presence of an analogous cell surface receptor for aldosterone in rabbit urinary bladder. The relationships between newly synthesised epithelial Na^+ channel subunits and intact Na^+ channels recycled from the apical cell membranes awaits further investigation.

Acknowledgements

This work was supported by Remedi and Research into Ageing. We are grateful also to C. Fuller and D. Benos (University of Alabama at Birmingham, USA) for the gift of antibodies to epithelial Na^+ channel subunits.

References

Bentley, P.J., 1966. The physiology of the urinary bladder of amphibia. Biol. Rev. 41, 275–316.

Burton, T.J., Edwardson, J.M., Ingham, J., Tempest, H.V., Ferguson, D.R., 2002. Regulation of Na^+ channel density at the apical surface of rabbit urinary bladder epithelium. Eur. J. Pharmacol. 448, 215–223.

Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D., Rossier, B.C., 1994. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. Nature 367, 463–467.

Crabbe, J., 1961. Stimulation of active sodium transport across the isolated toad bladder after injection of aldosterone to the animal. Endocrinology 69, 673–682.

Crabbe, J., de Weer, P., 1964. Action of aldosterone on the bladder and skin of the toad. Nature 202, 278–279.

Ecelbarger, C.A., Kim, G.-H., Terris, J., Masilamani, S., Mitchell, C., Reyes, I., Verbalis, J.G., Knepper, M.A., 2000. Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney. Am. J. Physiol. 279, F46–F53.

Edelman, I.S., Bogoroch, R., Porter, G.A., 1963. On the mechanism of action of aldosterone on sodium transport. The role of protein synthesis. Proc. Natl. Acad. Sci. U. S. A. 50, 1169–1177.

Evans, T., Smith, M.M., Tanner, L.I., Harden, T.K., 1984. Muscarinic cholinergic receptors of two cell lines that regulate cyclic AMP metabolism by different molecular mechanisms. Mol. Pharmacol. 26, 395–404.

Fagan, K.A., Mahey, R., Cooper, D.M.F., 1996. Functional co-localisation of transfected Ca^{2+} stimulable adenylyl cyclases with capacitative Ca^{2+} entry sites. J. Biol. Chem. 271, 12438–12444.

Ferguson, D.R., Kennedy, I., Burton, T.J., 1997. ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes—a possible sensory mechanism? J. Physiol. 505, 503–511.

Kurrasch, D.M., Huang, J., Wilkie, T.M., Repa, J.J., 2004. Quantitative real-time polymerase chain reaction measurement of regulators of G-protein signalling mRNA levels in mouse tissues. Methods Enzymol. 389, 3–15.

Lewis, S.A., Diamond, J.M., 1976. Na^+ transport by rabbit urinary bladder, a tight epithelium. J. Membr. Biol. 28, 1–40.

Lewis, S.A., Wills, N.K., 1983. Apical membrane permeability and kinetic properties of the sodium pump in rabbit urinary bladder. J. Physiol. 341, 169–184.

Renard, S., Voilley, N., Bassilana, F., Lazdunski, M., Barbry, P., 1995. Localisation and regulation by steroids of the alpha, beta and gamma subunits of the amiloride-sensitive Na^+ channel in colon, lung and kidney. Pflugers Arch. 430, 299–307.

Sheader, E.A., Wargent, E.T., Ashton, N., Balment, R.J., 2002. Rapid stimulation of cyclic AMP production by aldosterone in rat inner medullary collecting ducts. J. Endocrinol. 175, 343–347.

Wickham, J.E.A., 1964. Active transport of sodium by the mammalian bladder epithelium. Invest. Urol. 2, 145–153.