Volume 294, number 3, 171–174 FEBS 10454 December 1991

Receptor-mediated induction of aminopeptidase A (APA) of human glomerular epithelial cells (HGEC) by glucocorticoids

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Received 2 September 1991

Membrane-bound peptidases are critical regulators of peptide hormones. We therefore characterized aminopeptidase A (APA) activity in human glomerular epithelial cells (HGEC) and studied the control of its expression. APA, which splits off the N-terminal Asp from angiotensin II (AII), was present at the surface of HGECs (55% of the total enzyme). APA activity was calcium-dependent and was inhibited by amastatin. Treatment of HGECs by dexamethasone (DEX) increased ecto-APA activity in a dose- and time-dependent manner. Maximal increase of APA activity (× 2) occurred after treatment with 0.5 μM DEX for 5 days. Higher concentrations (1–10 μM) of aldosterone (ALD) stimulated APA activity to a lesser extent (× 1.25). Actinomycin D and cycloheximide prevented and RU 38486, a glucocorticoid receptor antagonist, suppressed the DEX-induced increase in APA activity. These results indicate that AII availability at glomerular receptor sites may be reduced by DEX and suggest a role for glucocorticoids in AII-dependent changes of glomerular filtration rate.

Aminopeptidase A; Glomerular epithelial cells; Glucocorticoids; Angiotensin II

1. INTRODUCTION

Aminopeptidase A (angiotensinase A, APA, L-α-aspartyl(L-α-glutamyl)-peptide hydrolase, EC 3.4.11.7) is a membrane-bound peptidase which modulates the physiological responses to angiotensin II (AII) and other peptides by degrading them to inactive metabolites. Vascular endothelial cells [1], placental microvilli [2], pre-B and immature B lymphocytes [3], and various regions of the kidney [4,5] are rich in APA activity. The cDNA encoding the BP-1/6C3 antigen, which is identical to APA, has been isolated and characterized in murine pre-B cells [6]. BP-1/6C3 expression was induced by interleukin 7 in pre-B cells but not in mature B-cells [7]. In the kidney, APA activity is high in the glomerulus, particularly in the podocytes, and in the proximal tubule, where its activity is mostly located in the brush border [4,5].

To evaluate the possible regulation of this enzyme, we investigated the effects of glucocorticoids on APA in human glomerular epithelial cells (HGEC). Aminopeptidase N (APN, EC 3.4.11.2), another epithelial cell surface peptidase, was studied in parallel. The results show that glucocorticoids induce APA expression in HGECs. Evidence is also presented that this effect occurs through a receptor-mediated mechanism.

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2. MATERIALS AND METHODS

2.1. *Materials*

Cell culture medium, antibiotics and other materials for culture were from Flow Laboratories (Irvine, UK). Cell culture Petri dishes and plates were from Nunc (Roskilde, Denmark) or Costar (Cambridge, MA). L-glutamic acid-4-nitroanilide was from Serve (Heidelberg, Germany); [^{3}H]thymidine (25 Ci/mmol) was from Dositek (Orsay, France); alanine *p*-nitroanilide, gly-pro-*p*-nitroanilide, dexamethasone, aldosterone, actinomycin D, cycloheximide and amastatin were from Sigma (St. Louis, MO). The glucocorticoid antagonist, RU 38486, was a gift from Roussel-Uclaf (Paris, France); angiotensin II (Asn^1, Val^5 AII) was donated by Ciba Geigy (Basel, Switzerland).

2.2. *Epithelial cell culture*

Isolation and characterization of HGEC were performed as previously described [8]. Glomeruli were prepared by differential sieving and centrifugation from human cadaver kidneys judged unsuitable for transplantation. A homogeneous population of epithelial cells was obtained by collagenase digestion of isolated glomeruli. These cells were cultured at 37°C in RPMI 1640 medium buffered with 29 mM HEPES to pH 7.4 and supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine under an atmosphere of 5% CO_2/95% air. HGEC reached confluence after 10–15 days. They were subcultured and studied after one to three passages.

2.3. *Enzyme assays*

Surface APA activity was determined on confluent cultures of HGEC in 24-well plates. Cells were rinsed three times and incubated at 37°C in 50 mM Tris-HCl buffer, pH 8.0, containing 130 mM NaCl and 10 mM $CaCl_2$. The enzymatic reaction was started by addition of 10 mM L-glutamic acid-α-4-nitroanilide. Incubation was carried out with gentle agitation for 10–30 min under zero-order kinetic conditions. The amount of *p*-nitroanilide formed was measured in the supernatant by reading at an OD of 405 nm. Cell-free and substrate-free blanks were run in parallel.

Surface APN activity was determined similarly but in phosphate-buffered saline, pH 7.8, containing NaCl (140 mM), $MgCl_2$ (1.0 mM),

Na_2HPO_4 (9 mM), NaH_2PO_4 (9 mM), KH_2PO_4 (1.5 mM), KCl (3 mM). Alanine *p*-nitroanilide (1.5 mM) was used as substrate. Dipeptidylpeptidase IV was assayed in a 50 mM Tris-HCl buffer, pH 8.0, containing NaCl (130 mM), $MgCl_2$ (1 mM) and gly-pro-*p*-nitroanilide (1.5 mM) as substrate. Total enzyme activity was measured in cells which were sonicated using a Branson sonicator (model W 185 D, Branson Sonic Power Co. Danbury, USA) at position 3. The tube containing the cells was immersed in iced water and the sample was sonicated for 60 s.

2.4. *Determination of cell protein and DNA synthesis*

Cell protein was estimasted according to Lowry et al. [9] using bovine serum albumin as standard. DNA synthesis was assessed using [^{3}H]thymidine incorporation. Cells were incubated with dexamethasone at increasing concentrations for 72 h. [^{3}H]thymidine (1 μCi/well) was added 17 h before harvesting. At the end of incubation, the cells were washed and TCA-precipitable radioactivity was measured by liquid scintillation spectroscopy.

2.5. *Statistical analysis*

Enzyme activity was expressed as nmol *p*-nitroanilide formed per minute and per mg of cell protein. Values are given as means ± SE. Comparisons between groups were analysed using Student's *t*-test and analysis of variance. Correlation between two parameters was estimated by regression analysis.

3. RESULTS

3.1. *Characterization of surface aminopeptidase A*

Surface APA activity was determined on viable cells in culture. Viability was determined by the Trypan blue exclusion method. The amount of substrate hydrolysed was linear over 10–30 min making it unlikely that a significant portion of substrate was hydrolysed after entering the cells. Most of the APA activity (55% of the total enzyme) was expressed at the cell surface. At a substrate concentrtaion of glutamyl *p*-nitroanilide of 1.5 mM, APA activity was inhibited by 43.1% with 1 mM A II. Aspartic and glutamic acid (10 mM) inhibited APA activity by 20.2% and 15.2%, respectively. Amastatin (30 μM), an inhibitor of aminopeptidases, reduced the activity of APA by 82.5%. Pretreatment of HGEC with EDTA (5 mM) and omission of divalent cations in the incubation medium reduced APA activity to 33% of that obtained in the presence of 1 mM $CaCl_2$.

3.2. *Induction of surface aminopeptidases*

APA, but not APN activity, increased significantly with time in cells exposed to 0.5 μM dexamethasone (Fig. 1). Stimulation was significant after 24 h incubation ($P < 0.01$) and increased progressively over the 5 days of study. Dose–response curves of APA and APN activities as a function of the concentration of dexamethasone in the incubation medium are shown in Fig. 2. APA activity increased significantly ($P < 0.01$) (1.8 times basal value) at 0.5 μM dexamethasone. Increasing concentrations of dexamethasone produced similar or slightly lower increases. APN activity was also stimulated by dexamethasone but to a lesser extent (1.14 times basal value at 0.5 μM) than for APA activity. In contrast with APA, APN stimulation increased progressively with dexamethasone concentration to reach a plateau at 5–10 μM. The stimulation of APA and APN activities was not the result of an effect of dexamethasone on cell growth since [^{3}H]thymidine incorporation was unchanged at concentrations between 0.1 and 5 μM and even diminished (− 34%) at 10 μM. Dose–response curves with aldosterone are presented on Fig. 3. Aldo-

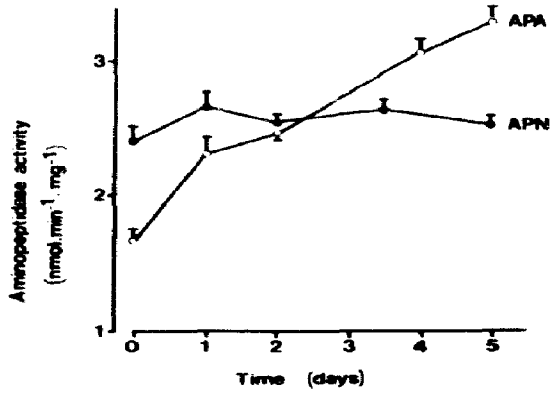

Fig. 1. Aminopeptidase A (open circles, APA) and aminopeptidase N (closed circles, APN) activities in the presence of 0.5 μM dexamethasone as a function of time. Means ± SE of 4 determinations are shown. Data were analysed by regression analysis. APA activity increases significantly with time ($r = + 0.95$, $P<0.001$). There is no significant correlation for APN.

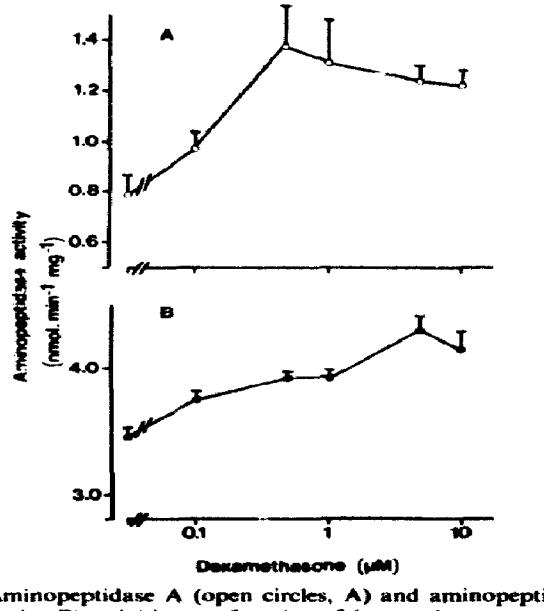

Fig. 2. Aminopeptidase A (open circles, A) and aminopeptidase N (closed circles, B) activities as a function of dexamethasone concentration. HGEC were cultured with dexamethasone for 72 h. Means ±SE of 4 determinations are shown. Data were analysed using Student's *t*-test. Aminopeptidase A and N activities at concentrations of dexamethasone equal to or higher than 0.5 μM are significantly greater than control ($P < 0.05$).

sterone stimulated APA activity but higher concentrations (1–10 μM) were required and stimulation was less (1.25 times basal value) than with dexamethasone. APN activity also increased with aldosterone treatment (1.34 times basal value). Surface HGEC dipeptidylpeptidase IV activity (DDP IV) remained unchanged after 72 h exposure to 0.1–10 μM dexamethasone (data not presented).

To evaluate whether the effect of dexamethasone on APA required synthesis of a new enzyme protein, HGEC were pretreated with actinomycin D, an inhibitor of RNA synthesis, or cycloheximide, an inhibitor of protein synthesis. Stimulation by dexamethasone was inhibited by both cycloheximide and actinomycin D. Receptor occupancy by RU 38486, a glucocorticoid receptor antagonist, also prevented APA induction by dexamethasone (Table I).

Dexamethasone stimulated both surface and total APA activities. Surface APA activity was 1.72 ± 0.14 and 3.25 ± 0.45 nmol·min^{-1}·mg^{-1} and total APA activity was 3.35 ± 0.55 and 5.89 ± 0.75 nmol·min^{-1}·mg^{-1} before and after dexamethasone treatment, respectively. The results were obtained with 3 batches of HGEC and with 3–8 determinations for each of them.

4. DISCUSSION

Dexamethasone at concentrations between 0.1 and 10 μM increased APA activity of HGEC with a maximal stimulation observed at 0.5 μM. This effect was time-dependent increasing steadily for up to 5 days of treatment. The increase in APA activity was prevented by pretreatment with actinomycin D and cycloheximide suggesting that transcription and enzyme synthesis are responsible for the increased activity. Direct demonstration by Northern blotting, cannot be performed since cDNA clones for human APA have not yet been isolated. RU 38486, a glucocorticoid receptor antagonist, also prevented the increase in APA showing thereby that the dexamethasone effect on APA activity is receptor-mediated. Aldosterone, a mineralocorticoid, also increased APA activity, but much less than dexamethasone and only at higher concentrations (5-10 μM). APA belongs to the family of peptidases which modulate the responses to various peptides mostly by degrading them to inactive metabolites. APA splits off the N-terminal Asp from A I and A II and regulates the action of the latter in the glomerulus and other tissues where expression of a local renin-angiotensin system has been demonstrated. APA has been cloned from murine pre-B cells [6]. However, little is known about the regulation of its gene.

Glucocorticoids can induce different proteins like angiotensin converting enzyme [10,11], neutral endopeptidase [12] or immunoglobulin E [13]. Induction of peptidases and increase in immunoglobulin E synthesis may represent mechanisms whereby these proteins are regulated under physiologic conditions and the therapeutic actions of glucocorticoids are mediated. Kidney APA regulation has not been extensively studied. APA is lo-

Table I

Effect of cycloheximide, actinomycin D and RU 38486 on dexamethasone-induced aminopeptidase A activity in human glomerular epithelial cells

Treatment	Aminopeptidase A activity (nmol·min^{-1}·mg^{-1})
Control	1.22 ± 0.03
Dexamethasone (0.5 μM)	1.87 ± 0.07
Cycloheximide (0.1 μg·ml^{-1})	1.11 ± 0.02
Dexamethasone (0.5 μM) + Cycloheximide (0.1 μg·ml^{-1})	1.24 ± 0.08
Actinomycin D (0.1 μg·ml^{-1})	1.04 ± 0.03
Dexamethasone (0.5 μM) + Actinomycin D (0.1 μg·ml^{-1})	1.19 ± 0.06
Control	1.45 ± 0.09
Dexamethasone (0.5 μM)	2.16 ± 0.07
RU 38486 (10 μM)	1.50 ± 0.04
Dexamethasone (0.5 μM) + RU 38486 (10 μM)	1.61 ± 0.03

Cells were preincubated with cycloheximide, actinomycin D or RU 38486 for 1 h before addition of dexamethasone and were then incubated for a further period of 48 h. Values are means ±SE of 4–8 determinations. Data were analysed using two-way analysis of variance for repeated data. The effect of dexamethasone was statistically significant ($P < 0.001$). Interactions between dexamethasone and each of the agents tested (cycloheximide, actinomycin, RU 38486) were also significant ($P < 0.001$).

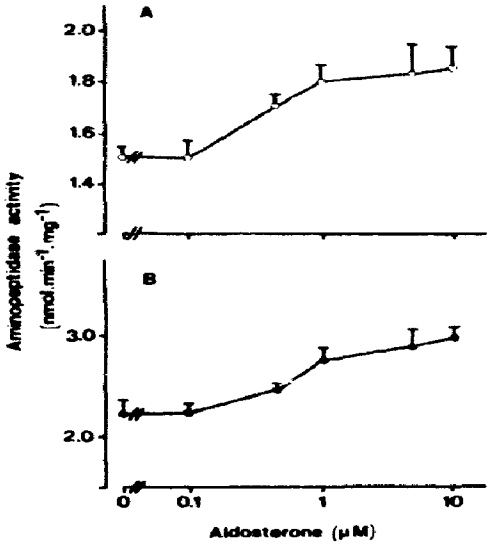

Fig. 3. Aminopeptidase A (open circles, A) and aminopeptidase N (closed circles, B) activities as a function of aldosterone concentration. HGEC were cultured with aldosterone for 72 h. Means ±SE of 4 determinations are shown. Data were analysed using Student's t-test. Aminopeptidase A and N activities were significantly greater than control values at concentrations equal to or higher than 0.5 μM and 1 μM, respectively.

calized in the epithelial and endothelial cells of the glomerular tuft and probably modulates glomerular functions by inactivating A II. In keeping with this possibility, Wolf et al. [14] suggested that glomerular APA might be involved in the early regulation of the intrarenal renin-angiotensin system and modify glomerular adaptations after renal mass ablation.

Several peptidases which metabolize A I and A II are present in the kidney [15]. The results of the present study indicate that glomerular receptor site availability of A II is controlled by APA.

This is the first demonstration that glucocorticoids control the expression of APA in HGEC. The effect is specific for glucocorticoids and was obtained with physiologic concentrations of dexamethasone (0.5 μM) suggesting that the control of APA expression can be influenced by normal corticoid secretion as well as by pharmacologic drug administration. Moreover, it may well be that A II-dependent changes in glomerular filtration rate are modulated by glucocorticoids.

REFERENCES

[1] Johnson, A.R. and Erdös, E.G. (1977) J. Clin. Invest. 59, 684–695.
[2] Johnson, A.R., Skidgel, R.A., Gafford, J.T. and Erdös, E.G. (1984) Peptides 5, 789–796.
[3] Wu, Q., Li, L., Cooper, M.D., Pierres, M. and Gorvel, J.P. (1991) Proc. Natl. Acad. Sci. USA 88, 676–680.
[4] Kugler, P. (1981) Histochemistry 72, 269–278.
[5] Kugler, P., Wolf, G. and Scherberich, J. (1985) Histochemistry 83, 337–341.
[6] Wu, Q., Lahti, J.M., Air, G.M., Burrows, P.D. and Cooper, M.D. (1990) Proc. Natl. Acad. Sci. USA 87, 993–997.
[7] Sherwood, P.J. and Weissman, I.L. (1990) Int. Immunol. 2, 399–406.
[8] Ardaillou, N., Nivez, M.P., Striker, G. and Ardaillou, R. (1983) Prostaglandins 26, 773–784.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[10] Friedland, J., Setton, C. and Silverstein, E. (1977) Science. 197, 64–65.
[11] Mendelsohn, F.A.O., Lloyd, C.J., Kachel, C. and Funder, J.W. (1982) J. Clin. Invest. 70, 684–692.
[12] Borson, D.B. and Gruenert, D.C. (1991) Am. J. Physiol. 260, L83–L89.
[13] Wu, C.Y., Sarfati, M., Heusser, C., Fournier, S., Rubio-Trujillo, M., Peleman, F. and Delespesse, G. (1991) J. Clin. Invest. 87, 870–877.
[14] Wolf, G., Thaiss, F., Scherberich, J.E., Shoeppe, W. and Stahl, R.A.K. (1990) Kidney Int. 38, 862–868.
[15] Erdös, E.G. and Skidgel, R.A. (1990) Kidney Int. 30, S24–S27.