Br. J. Pharmac. (1984), **82**, 891–895

Location of [^{125}I]-angiotensin II receptors on rat kidney cortex epithelial cells

Helen M. Cox, Kenneth A. Munday & Judith A. Poat

Department of Physiology & Pharmacology, Medical & Biological Sciences Building, University of Southampton, Bassett Crescent East, Southampton SO9 3TU

1 Angiotensin II (AII) stimulates active Na^+ extrusion from Na^+ loaded renal cortex slices.

2 Specific high affinity [^{125}I]-AII binding sites in partially purified basolateral and brush-border epithelial membranes exhibit a K_D of 0.88 nM and B_{max} of 321.13 fmol mg^{-1} protein.

3 Separation and purification of brush-border membranes yielded high affinity [^{125}I]-AII binding sites with K_D of 1.02 nM and B_{max} of 56.6 fmol mg^{-1} protein.

4 Angiotensin II receptors of the same affinity are present on renal cortex brush-border and basolateral membranes but a greater proportion are located on the latter. These receptors may be involved in the direct control of Na^+ and water transport by AII.

Introduction

Angiotensin II (AII) plays a major role in the maintenance of homeostasis via both central and peripheral actions. Of the peripheral actions perhaps the least well understood is the mechanism by which AII stimulates salt and water re-absorption across transporting epithelia, particularly in the renal cortex. The synthesis and storage of renin within juxtaglomular cells (Granger *et al.*, 1972) and the high concentration of converting enzyme present on the brush-borders of the proximal tubule epithelia (Ward *et al.*, 1975) strongly suggest an intrarenal site for AII production. It is likely therefore, that a degree of AII's antinatriuretic and antidiuretic effects are due to a direct action of intrarenally produced hormone. AII appears to have direct actions on renal transporting epithelia (Munday *et al.*, 1972; Harris & Young, 1977) and also plays a role in the control of glomerular filtration rate (Bonjour & Malvin, 1969; Arundell & Johns, 1982) therefore, indirectly affecting ion absorption. Constriction of the efferent and afferent arterioles by AII reduces renal blood flow and as a result water and electrolyte excretion is decreased.

The identification of specific binding sites for [^{125}I]-AII in partially purified basolateral/brush-border membranes prepared from renal cortex epithelia provides evidence in support of a direct hormone action (Cox *et al.*, 1983a). These high affinity binding sites had similar characteristics to AII receptors located in adrenal cortex (Glossmann *et al.*, 1974) and mesenteric artery membranes (Gunther *et al.*, 1980). Specific [^{125}I]-AII binding in renal cortex membranes exhibited partial sodium dependence and was modulated by guanine nucleotides (Cox *et al.*, 1983b). Furthermore, a good correlation between the ability of AII analogues displacing specific [^{125}I]-AII binding and their ability to stimulate active sodium extrusion from Na^+ loaded kidney cortex slices has been shown to exist (Boland *et al.*, 1983).

A recent study (Brown & Douglas, 1982) identified high affinity specific [^{125}I]-AII binding sites on renal cortex brush-border membranes. It was suggested that these sites may be involved in contraction of microvilli smooth muscle resulting in an increased solute flow (Kenny & Booth, 1978). Alternatively AII was postulated to bind to a transport molecule present in brush-borders, thereby influencing ion transport. In view of this and other information it seems important to examine the distribution of specific [^{125}I]-AII binding sites on renal cortex epithelial cell membranes and this paper presents such a study.

Methods

Membrane preparation and binding procedure

Male Wistar rats (250–300 g) were killed by cervical dislocation and the kidneys removed immediately. Renal cortex tissue was rapidly dissected on ice and homogenized in a tight fitting homogenizer (0.178 mm clearance) in 20 volumes of 2 mM Tris-

HCl buffer, pH 7.0, containing 50 mM mannitol. Calcium chloride (at a final concentration of 10 mM) was added to precipitate basolateral and intracellular membranes and the homogenate was stirred for 20 min on ice. Renal cortex brush-border membranes were then isolated by differential centrifugation according to Will & Hopfer (1979), using a Beckman J2-21 centrifuge (2–4°C). All membrane fractions used for either receptor binding studies or enzyme assays were resuspended in 0.1 M D-mannitol, 1 mM Tris/HEPES, pH 7.5.

The preparation of a basolateral/brush-border membrane fraction was obtained from rat renal cortex following the procedure of Heidrich *et al.*, (1972). Renal cortex tissue was homogenized in a loose fitting homogenizer (0.43 mm clearance) in a 10% (w/v) 250 mM sucrose solution containing 10 mM triethanolamine HCl and 0.1 mM phenylmethylsulphonylfluoride (PMSF) adjusted to pH 7.6. Epithelial membranes were then prepared by differential centrifugation according to the original authors.

Membrane protein (20–300 μg per tube) was incubated with 0.7–0.8 nM [^{125}I]-angiotensin II (specific activity 1880 Ci mmol^{-1}) for 5 min at 22°C in 20 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM Na_2 EDTA, 0.1 mM PMSF and 0.2% bovine serum albumin (final volume 250 μl). Specific binding was determined by the addition of 1 μM unlabelled angiotensin II. Incubations were terminated by cooling; free and bound ligand were separated by addition of 10 ml of ice cold buffer, pH 7.4 with filtration through Whatman GF/B filters under vacuum. Filter blanks were determined routinely and radioactivity counted in a Beckman Biogamma counter.

Enzyme assays

The degree of separation of basolateral and brush-border membranes was assessed by assaying ouabain sensitive Na^+ K^+-ATPase (Lewis *et al.*, 1975) and alkaline phosphatase (Eicholz & Crane, 1965) activities, respectively. Enzyme activities are expressed as μmol of either inorganic phosphate (P_i) or paranitrophenol (PNP) produced per min per mg of protein where protein levels were determined using Coomassie brilliant blue dye (Bradford, 1976).

Materials

Monoiodinated [^{125}I]-angiotensin II (specific activity 1880 Ci mmol^{-1}) was purchased from N.E.N., Boston, MA, U.S.A.. Unlabelled angiotensin II (AII) was obtained from Sigma, Poole and all other compounds were of analytical grade.

Results

Alkaline phosphatase specific activity was increased 9 fold in the purified brush-border fraction, P6 (3.84 ± 0.4 μmol PNP mg^{-1} protein min^{-1}) compared with the initial crude renal cortex homogenate (0.43 ± 0.02 μmol PNP mg^{-1} protein min^{-1}). In contrast ouabain-sensitive Na^+ K^+-ATPase activity was reduced 4 fold in P6 (0.005 ± 0.0002 μmol P_i mg^{-1} min^{-1}) compared with the starting material (0.018 ± 0.0002 μmol P_i mg^{-1} min^{-1}) indicating that the final membrane fraction is composed predominantly of brush-border membranes. Total recovery of alkaline phosphatase activity was obtained whereas only 46% of the ouabain-sensitive Na^+ K^+-ATPase activity was recovered from the original homogenate. The enzyme profiles obtained during brush-border membrane preparation were very similar to those described by Will & Hopfer (1979).

Specific [^{125}I]-AII binding was recorded in each of the fractions obtained during differential centrifugation. Some enrichment of binding was obtained in P2 (40.8 ± 5.8 fmol mg^{-1} protein) and S3 (44.6 ± 7.4 fmol mg^{-1} protein) following calcium precipitation of basolateral and intracellular membranes (P1). However, the specific [^{125}I]-AII binding in P6 (21.6 ± 5.3 fmol mg^{-1}) was not significantly different from that found in the starting material (18.5 ± 1.4 fmol mg^{-1}). No specific binding was detected in three fractions normally discarded, S4, P5 or S6. Na^+ K^+-ATPase activity was detected in low levels in P5 (0.011 ± 0.001 μmol P_i mg^{-1} min^{-1}) and was not detectable in S4 or S6. Of the fractions normally discarded P1 had the greatest total number of binding sites ($13,897 \pm 1,412$ fmol). This pellet, obtained immediately after the incubation of renal cortex homogenate with 10 mM $CaCl_2$, exhibited three times the Na^+ K^+-ATPase activity (0.014 ± 0.002 μmol P_i mg^{-1} min^{-1}) and only 15% of alkaline phosphatase activity (0.59 ± 0.026 μmol PNP mg^{-1} min^{-1}) found in P6. This suggests that P1 is composed of proportionally more basolateral than brush-border membranes, together with intracellular membranes (Will & Hopfer, 1979). This pellet (P1) also contained 217 times the number of [^{125}I]-AII binding sites obtained with the purified brush-border fraction, P6 (64.0 ± 15.7 fmol). The enrichment of alkaline phosphatase, Na^+ K^+-ATPase and specific [^{125}I]-AII binding in the fractions not discarded during purification of renal cortex brush-border membranes are shown in Figure 1a and the levels of total specific [^{125}I]-AII binding in each fraction in Figure 1b. The enrichment profile obtained for [^{125}I]-AII binding in each fraction more closely parallels the enrichment of Na^+ K^+-ATPase rather than alkaline phosphatase. Taking these and the previous results into account, one may presume that the basolateral

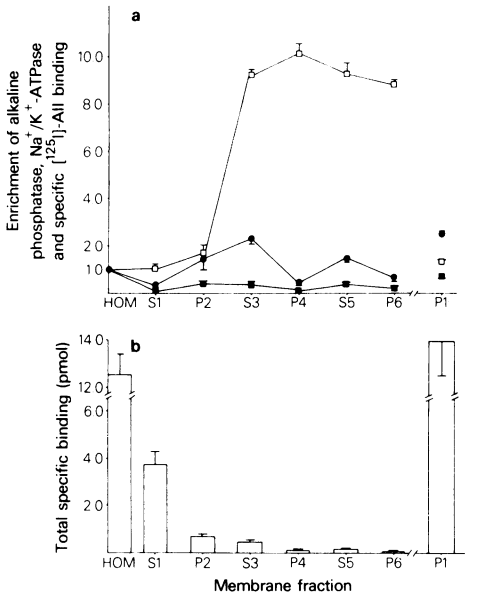

Figure 1 (a) Enrichment of Na^+ K^+-ATPase (■; $n=2$), alkaline phosphatase (□; $n=3$) and specific [^{125}I]-angiotensin II (AII) binding (●; $n=4$) in fractions produced during the preparation of purified renal cortex brush-border membranes. Each point represents the mean from 2–4 experiments (see n values) performed in triplicate. Vertical lines show 1 s.e.mean. (b) Total binding obtained in each fraction used in the preparation of brush-border membranes (P6). P1 is also included here for comparison although this fraction was normally discarded. Each column represents the mean, and vertical lines show 1 s.e.mean from a single representative experiment performed in triplicate.

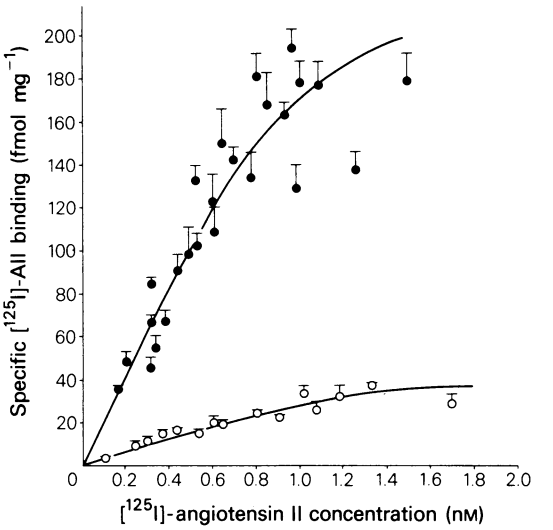

Figure 2 Comparison of saturable specific [^{125}I]-angiotensin II (AII) binding in basolateral/brush-border membranes (●) and purified brush-border membranes (○) from rat renal cortex. Fitting binding data to the Michaelis-Menten equation resulted in a K_D of 0.88 nM and B_{max} of 321.1 fmol mg^{-1} in the combined membrane fraction compared and a K_D of 1.02 nM and B_{max} of 56.6 fmol mg^{-1} in the purified brush-border fraction. Each point represents the mean and vertical lines show 1 s.e.mean.

membranes of renal cortex epithelia contain far greater numbers of AII binding sites than the brush-border membranes.

Additional evidence for this proposal was obtained from the comparison of saturable specific [^{125}I]-AII binding obtained in P6 with that exhibited by a membrane fraction composed of both basolateral and brush-border membranes (Heidrich *et al.*, 1972). The final membrane fraction obtained by this latter method displayed a 14 fold enrichment of alkaline phosphatase activity (0.42 ± 0.05 to 5.68 ± 0.49 μmol PNP mg^{-1} protein min^{-1}) and 6 fold enrichment (0.02 ± 0.01 to 0.13 ± 0.02 μmol P_i mg^{-1} protein min^{-1}) of ouabain sensitive Na^+ K^+-ATPase activity (Cox *et al.*, 1983a). These increased enzyme activities indicated purification of both brush-border and basolateral membranes and electron microscopic investigation of the final fraction confirmed this.

Figure 2 shows the marked difference in the maximum number of binding sites obtained in the combined basolateral/brush-border fraction compared with purified brush-border membranes. Saturation analysis, achieved by fitting binding data to the Michaelis-Menten equation yielded similar affinity constants (K_D) in the two membrane fractions (0.88 nM in the basolateral/brush-border fraction and 1.02 nM in purified brush-borders). In contrast the maximal binding capacity obtained with the combined membrane fraction ($B_{max} = 321.13$ fmol mg^{-1}) was 5.7 times greater than the B_{max} (56.6 fmol mg^{-1}) obtained with purified brush-border membranes. [^{125}I]-AII specific binding sites in renal cortex epithelial membranes therefore appear to exhibit the same affinity. However, a greater proportion of the sites are located on the basolateral membranes.

Discussion

Purification of renal cortex brush-border membranes was achieved using the calcium precipitation method of Will & Hopfer (1979). The final fraction was enriched 9 fold with alkaline phosphatase activity and depleted of ouabain-sensitive Na^+ K^+-ATPase activity. These results confirm the observations of Brown & Douglas (1982) who, using the same membrane preparation, also identified specific [^{125}I]-AII

binding sites. However, on examination of specific [^{125}I]-AII binding to fractions produced during the isolation procedure the highest concentration of binding sites exists in the material precipitated by calcium (P1). Total [^{125}I]-AII binding was 13,897.2 ± 1,411.9 fmol compared with only 64.0 ± 15.7 fmol in P6, the brush-border fraction. The enzyme marker activities suggest that P1 contains basolateral membranes as described by Will & Hopfer (1979). The possible presence of glomeruli, mesangial cells and blood vessel fragments cannot be excluded from P1. These structures have been shown to contain high affinity AII receptors (Sraer *et al.*, 1974; Foidart *et al.*, 1980; Caldicott *et al.*, 1981) and their presence may contribute significantly to the binding level exhibited by P1. Nevertheless, it is clear that a larger proportion of specific [^{125}I]-AII binding sites are present on the renal cortex basolateral membranes. This was substantiated by the comparison of saturation binding data from purified brush-border membranes with that of a combined fraction of basolateral/brush-border membranes. The latter fraction was originally shown not to be contaminated by glomeruli, vascular or connective tissue (Heidrich *et al.*, 1972). Specific [^{125}]-AII binding to brush-border membranes alone exhibited a K_D of 1.02 nM and B_{max} of 56.6 fmol mg^{-1} protein compared with a K_D of 0.88 nM and B_{max} of 321.13 fmol mg^{-1} protein obtained with the basolateral/brush-border membrane fraction. Renal cortex epithelial [^{125}I]-AII binding sites therefore possess the same affinity and a greater proportion of the sites appear to be located on the basolateral membrane.

This conclusion is somewhat at variance with a second study by Brown & Douglas (1983). They found no significant difference between the number of binding sites on basolateral and brush-border membranes. The maximal binding capacity quoted by these authors for brush-border membranes was 10 times that obtained in this study. There is no obvious explanation for such a discrepancy although differences in buffer composition and in methodology may account in part for the variation in results. Such a large difference cannot be due to experimental inconsistencies alone. Brown & Douglas (1983) also found no significant difference between the affinity of the binding sites on basolateral and brush-border membranes. This is in agreement with the results of this study.

In conclusion, renal tubular AII receptors are predominantly located on basolateral membranes and these receptors may be involved in the direct control of renal salt and water transport by locally generated, blood borne AII.

References

ARUNDELL, L.A. & JOHNS, E.J. (1982). Effect of converting enzyme inhibition on the renal haemodynamic responses to noradrenaline infusion in the rat. *Br. J. Pharmac.*, **75**, 553–558.

BONJOUR, J.P. & MALVIN, R.L. (1969). Renal extraction of PAH, G.F.R. and $U_{Na}V$ in the rat during infusion of angiotensin. *Am. J. Physiol.*, **216**, 554–558.

BOLAND, O., COX, H.M., PARSONS, B.J. & POAT, J.A. (1983). Correlation of binding potencies and physiological responses of angiotensin analogues on rat kidney. *J. Physiol.*, **334**, 63–64P.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

BROWN, G.P. & DOUGLAS, J.G. (1982). Angiotensin II binding sites on isolated rat renal brush-border membranes. *Endocrinology*, **111**, 1830–1836.

BROWN, G.P. & DOUGLAS, J.G. (1983). Angiotensin II binding sites in rat and primate isolated renal tubular basolateral membranes. *Endocrinology*, **112**, 2007–2014.

CALDICOTT, W.J.H., TAUB, K.J., MARGULIES, S.S. & HOLLENBERG, N.K. (1981). Angiotensin receptors in glomeruli differ from those in renal arterioles. Kidney Int., **19**, 687–693.

COX, H.M., MUNDAY, K.A. & POAT, J.A. (1983a). The binding of [^{125}I]-angiotensin II to renal epithelial cell membranes. *Br. J. Pharmac.*, **79**, 63–71.

COX, H.M., POAT, J.A. & MUNDAY, K.A. (1983b). The effect of guanine nucleotides on [^{125}I]-angiotensin binding in rat kidney cortex epithelial membranes. *Biochem. Pharmac.*, **32**, 3601–3604.

EICHOLZ, A. & CRANE, R.K. (1965). Studies on the organisation of the brush-border in intestinal epithelial cells. *J. cell Biol.*, **26**, 687–691.

FOIDART, J., SRAER, J., DELARUE, F., MAHIEU, P. & ARDAILLOU, R. (1980). Evidence for mesangial glomerular receptors for angiotensin II linked to mesangial cell contractility. *FEBS Letts.*, **121**, 333–339.

GLOSSMANN, H., BAUKAL, A.J. & CATT, K.J. (1974). Properties of angiotensin II receptors in the bovine and rat adrenal cortex. *J. biol. Chem.*, **249**, 825–834.

GRANGER, P., DOLHEIM, H. & THURAU, K. (1972). Enzyme activities of the single juxtaglomerular apparatus in the rat kidney. *Kidney Int.*, **1**, 78–88.

GUNTHER, S., GIMBORNE, M.A. & ALEXANDER, R.W. (1980). Identification and characterisation of the high affinity vascular angiotensin II receptors in rat mesenteric artery. *Circulation Res.*, **47**, 278–286.

HARRIS, P.J. & YOUNG, J.A. (1977). Dose dependent stimulation and inhibition of maximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pflügers Arch.*, **367**, 295–297.

HEIDRICH, H.G., KINNE, R., KINNE-SAFFRAN, E. & HANNIG, K. (1972). The polarity of the proximal tubule cell in rat kidney. *J. cell Biol.*, **54**, 232–245.

KENNY, A.J. & BOOTH, A.G. (1978). Microvilli: their ultra-

structure, enzymology and molecular organisation. *Essays Biochem.*, **14,** 1–44.

LEWIS, B.A., ELKIN, A., MICHELL, R.H. & COLEMAN, R. (1975). Basolateral plasma membranes of intestinal epithelial cells. *Biochem. J..*, **152,** 71–84.

MUNDAY, K.A., PARSONS, B.J. & POAT, J.A. (1972). Studies on the mechanism of action of angiotensin II on ion transport by kidney cortex slices. *J. Physiol.*, **224,** 195–206.

SRAER, J.D., SRAER, J., ARDAILLOU, R. & MIMOUNE, O. (1974). Evidence for renal glomerular receptors for angiotensin II. *Kidney Int.*, **6,** 241–246.

WARD, P.E., GEDNEY, C.D., DOWBEN, R.M. & ERDOS, E.G. (1975). Isolation of membrane bound renal kallikrein and kininase. *Biochem, J.*, **151,** 755–758.

WILL, P.C. & HOPFER, U. (1979). Apparent inhibition of active non-electrolyte transport by an increased sodium permeability of the plasma membranes. *J. biol. Chem.*, **254,** 3806–3811.

(*Received February 21, 1984.*
Revised March 16, 1984.)