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SPECIAL REPORT

Modulation by locally produced luminal angiotensin II of proximal tubular sodium reabsorption via an AT_1 receptor

Siriphun Hiranyachattada & [1]Peter J. Harris

Department of Physiology, The University of Melbourne, Parkville, Victoria 3052, Australia

The concentration of angiotensin II reported in proximal tubular fluid in anaesthetized rats is considerably higher than in plasma, indicating secretion of this peptide into the tubular lumen. Shrinking split-drop micropuncture was used to examine the effect of endogenous angiotensin on sodium and water absorption in the proximal convoluted tubule. Addition of losartan, a nonpeptide AT_1 receptor blocker, to intratubular fluid increased fluid uptake by $15.7 \pm 3.9\%$ (10^{-5} M) and $24.7 \pm 9.4\%$ (10^{-4} M) whereas the AT_2 inhibitor, PD123319 had no effect. We conclude that angiotensin II is secreted into proximal tubular fluid and, in the anaesthetized rat, is maintained at a concentration that inhibits sodium and water transport via AT_1 receptors.

Keywords: Angiotensin II; micropuncture; proximal tubule; sodium transport; losartan; AT_1 receptors

Introduction Micropuncture experiments indicate that the concentration of angiotensin II in proximal tubular fluid considerably exceeds that in the filtrate (Seikaly *et al.*, 1990; Braam *et al.*, 1993) and it has been concluded that angiotensin II is secreted into the lumen at a site downstream from the glomerulus. Investigations of the luminal actions of angiotensin *in vivo* (Harris & Young, 1977; Wang & Chan, 1990) and *in vitro* (Li *et al.*, 1994) have indicated that picomolar to nanomolar concentrations of angiotensin II stimulate sodium and bicarbonate transport whereas micromolar concentrations cause inhibition. We have used nonpeptide angiotensin receptor blockers to examine whether endogenously produced angiotensin II in the proximal tubular lumen exerts a modulatory influence upon fluid absorption in the anaesthetized rat.

Methods Sprague-Dawley rats were anaesthetized with Inactin (110 mg kg^{-1} body weight), infused with 0.9% NaCl (1.6 ml h^{-1} 100 g^{-1}) and prepared for micropuncture as described previously (Harris *et al.*, 1987). A droplet of castor oil stained with Sudan Black was placed in the tubule through one barrel of a double-barrelled micropipette. A solution (intratubular fluid) of similar composition to mid-proximal tubule fluid (mmol kg^{-1}: NaCl 145, $NaHCO_3$ 5, KCl 5, $CaCl_2 2H_2O$ 1.5) was then injected from the other barrel to split the oil column. The rate of shrinking of the split droplet was determined by digital computer analysis of successive video frames captured at 1 s intervals (Harris *et al.*, 1987). After a 60 min equilibration period, proximal fluid uptake rate per unit surface area of epithelium (Jv_a) was determined in 3-5 tubules and a mean value calculated. Jv_a was then determined in a further 3 or more tubules using intra-tubular fluid containing nonpeptide AT_1 (losartan) or AT_2 (PD123319) inhibitors. Separate groups of animals were used to test the effects of each concentration of losartan or PD123319, such that each animal received only one dose of one inhibitor.

Results When added to the intratubular solution at 10^{-5} M, losartan increased mean proximal fluid absorption from $2.43 \pm 0.14 \times 10^{-4}$ $mm^3 mm^{-2} s^{-1}$ (control) to $2.80 \pm 0.14 \times 10^{-4}$ $mm^3 mm^{-2} s^{-1}$, ($n = 7$, $P < 0.005$, paired t test). At 10^{-4} M, a similar effect was observed (control: $2.21 \pm 0.13 \times 10^{-4}$ $mm^3 mm^{-2} s^{-1}$; losartan: $2.66 \pm 0.09 \times 10^{-4}$ $mm^3 mm^{-2} s^{-1}$; $n = 10$, $P < 0.05$). There was no effect of losartan at a lower concentration (10^{-6} M). The percentage changes in fluid absorption due to intratubular administration of losartan are shown in Figure 1. In a further series of experiments, addition of the AT_2 receptor antagonist, PD123319 (10^{-5} M) to intratubular fluid did not alter the mean rate of fluid uptake (control: $2.48 \pm 0.15 \times 10^{-4}$ $mm^3 mm^{-2} s^{-1}$; PD123319: $2.46 \pm 0.14 \times 10^{-4}$ $mm^3 mm^{-2} s^{-1}$; $n = 5$, NS).

Discussion The data indicate that angiotensin II is secreted into proximal tubular fluid and influences sodium and water uptake via AT_1 angiotensin receptors. Angiotensin II appears to be secreted rapidly since the period of approximately 60 s required for introduction and recording of the shrinking split-drop was sufficient to allow expression of losartan sensitivity.

The specificity and lack of agonist activity of losartan have resulted in this compound being used to define the sub-class of angiotensin receptors known as AT_1, or losartan-sensitive sites and also revealed the presence in many tissues of AT_2 angiotensin binding sites that are resistant to losartan and its active metabolite EXP3174 (Smith *et al.*, 1992). Autoradiographic studies of [^{125}I]-[Sar^1, Ile^8] angiotensin II binding *in vitro* indicated that in rat kidney approximately 90% of binding to all structures was associated with AT_1 sites. However, 15-20% of proximal tubular binding was displaced by a specific AT_2 an-

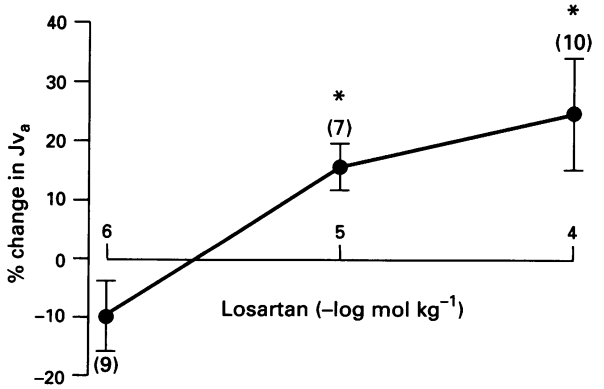

Figure 1 The effect of intratubular losartan on proximal tubular fluid absorption. Data are shown as means ± s.e.mean and presented as percentage changes in fluid absorption compared with control. Figures in parentheses indicate numbers of animals. *$P < 0.05$ (paired t test).

[1] Author for correspondence.

tagonist indicating the presence of this receptor sub-type (Zhuo *et al.*, 1993). In these experiments addition of the AT_2 inhibitor, PD123319 (Dudley *et al.*, 1990) to the artificial intratubular fluid did not affect fluid uptake and we infer that there is no substantial contribution of this receptor sub-type to the autocrine modulation of proximal fluid transport by luminal angiotensin II.

Direct measurements of angiotensin II concentrations in collected proximal tubular fluid have given values varying between 10^{-8} M (Braam *et al.*, 1993) and 4×10^{-8} M (Seikaly *et al.*, 1990). Free-flow micropuncture studies (Wang & Chan, 1990) indicate that sodium transport would be under an inhibitory influence of luminal angiotensin II at these concentrations. A substantial decrease in receptor occupancy following blockade of AT_1 receptors by losartan would therefore lead to increased absorption since concentrations of luminal angiotensin II between 10^{-12} M and 10^{-10} M are known to stimulate sodium transport.

We conclude that, in the anaesthetized rat, luminal angiotensin II is maintained at a concentration that inhibits sodium transport. The physiological significance of the maintenance of a high luminal concentration of this peptide is not yet clear but the observation has important implications for the design and interpretation of micropuncture studies of this type. Transepithelial fluid transport rates measured in previous split-drop experiments could have been influenced by accumulation of angiotensin II within the droplet. We propose that when it is necessary to measure basal levels of fluid uptake from proximal tubules the intratubular perfusate should contain 10^{-5} M losartan or an equivalent AT_1 receptor blocker.

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