Effects of angiotensin II receptor blockade on proximal fluid uptake in the rat kidney

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1 Angiotensin II has a well described dose-dependent biphasic action on proximal tubule fluid uptake, although the concentration and effect of endogenous luminal angiotensin II remain controversial.

2 Shrinking split-droplet micropuncture was used to examine the fluid uptake in response to the luminal application of three AT₁ antagonists (losartan, EXP3174, candesartan).

3 Addition of losartan at 10⁻⁸ M decreased fluid uptake rate (J_{v_a}) by 17.5 ± 2.2% (*P* < 0.05). Luminal addition of EXP3174 at concentrations between 10⁻⁹–10⁻⁵ M caused a dose-dependent decrease in fluid uptake, with a maximum decrease of 41.0 ± 9.5% (*P* < 0.01) at 10⁻⁶ M. Candesartan also decreased fluid uptake, by 21.9 ± 4.9% (*P* < 0.05) at 10⁻⁸ M and 23.6 ± 5.5% (*P* < 0.05) at 10⁻⁵ M.

4 All three antagonists at a low concentration (10⁻⁸ M) decreased fluid uptake. EXP3174 and candesartan at a higher concentration (10⁻⁵ M) also decreased fluid uptake in contrast to the previously reported effect of losartan.

5 We conclude that the endogenous concentration of angiotensin II in the proximal luminal fluid is low and exerts a stimulatory effect on fluid absorption. Losartan at concentrations greater than 10⁻⁶ M may have a non-selective action on fluid uptake.

Keywords: Angiotensin II; losartan; EXP3174; candesartan; fluid uptake; proximal tubule; kidney

Introduction

The addition of angiotensin II to either the peritubular capillary or the proximal lumen reveals a dose-dependent biphasic effect on fluid absorption. Angiotensin II at low concentrations (10⁻¹²–10⁻¹⁰ M) stimulates fluid uptake, while high concentrations (10⁻⁷–10⁻⁵ M) inhibit fluid uptake (Harris & Young, 1977; Schuster *et al.*, 1984; Wang & Chan, 1990; Li *et al.*, 1994). This biphasic response to angiotensin II indicates that sodium and water uptake will either be enhanced or suppressed depending upon the peritubular capillary and luminal concentrations of angiotensin II in the capillary and tubule lumen.

The endogenous concentration of intrarenal angiotensin II and its consequent action on fluid uptake in the proximal tubule lumen is uncertain. Samples of luminal fluid obtained by micropuncture from superficial proximal tubules have been shown to contain a nanomolar concentration of angiotensin II (Seikaly *et al.*, 1990; Braam *et al.*, 1993; Mitchell *et al.*, 1997). Previous studies in our laboratory (Hiranyachattada & Harris, 1996) and by Leyssac *et al.* (1997) concluded that addition of a high concentration of the AT₁ antagonist losartan (10⁻⁵ M) to the proximal tubule lumen enhanced fluid uptake. Taken together these observations support the existence of high endogenous concentrations of angiotensin II within the luminal fluid, and suggest that, at least in the anaesthetized rat, fluid uptake is suppressed by the endogenous concentration of angiotensin II in the lumen.

The presence of a high luminal AII concentration remains controversial because of a number of contradictory observations. Luminal additions of angiotensin II have been shown to have marked stimulatory effect on fluid uptake (Wang & Chan,

1990; Li *et al.*, 1994) that would not be expected if the endogenous angiotensin II concentration was already high. Quan & Baum (1996), who measured proximal tubule fluid uptake following luminal addition of a lower dose of losartan (10⁻⁸ M), observed a 35% decrease in uptake, adding further to the evidence favouring a low endogenous luminal angiotensin II concentration.

The primary purpose of the present study was to assess the influence of the endogenous concentration of angiotensin II by measuring the effects of angiotensin II receptor blockade on fluid absorption in proximal tubules. We used the AT₁ receptor antagonists, losartan, EXP3174 (a potent active metabolite of losartan) and candesartan, as the predominant angiotensin receptor in the proximal tubule has been reported to be the AT₁ subtype (Sechi *et al.*, 1992). A previous study from this laboratory supports this conclusion since AT₂ receptor blockade with PD-12319 did not affect fluid uptake (Hiranyachattada & Harris, 1996).

In addition, the proposal that losartan has a biphasic dose-dependent effect on fluid uptake was examined, since such a response pattern would be a key factor in interpreting the effects of pharmacological blockade of angiotensin II in the lumen.

Methods

Male Sprague-Dawley rats (300 ± 50 g; *n* = 68) were anaesthetized with Inactin (100 mg kg⁻¹ i.p.) and infused intravenously with 0.9% NaCl at 1.6 ml h⁻¹ 100 g bodyweight wt⁻¹. A carotid cannula was inserted to measure the blood pressure, which was continuously recorded on a chart recorder (Rikadenki, Model R-02). The left kidney was prepared for

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micropuncture as previously reported (Harris *et al.*, 1987). After a 1 h equilibration period, shrinking split-drop micropuncture was performed in midproximal convoluted tubule segments visible on the kidney surface. From a double-barreled micropipette Sudan black-stained castor oil was infused into the proximal tubule. Artificial tubular fluid (ATF) (in mM) NaCl 145, NaHCO₃ 5, KCl 5 and CaCl₂ 1.5, pH 7.4 was then injected from the other barrel of the pipette to split the oil column. Proximal fluid absorption was determined by the rate of change of volume of the shrinking drop and expressed per unit surface area of epithelium (J_{v_a}). The volume of the shrinking drop was measured using digital analysis of oil-water menisci in successive video frames taken at 1 s intervals. The experiment was terminated if the mean arterial blood pressure of the rat did not remain above 100 mmHg during the measurement period. Fluid uptake rate was determined in 3–5 tubules receiving a vehicle solution (ATF) and then in another 3–5 tubules following introduction of an AT₁ antagonist {losartan, (2-n-butyl-4-chloro-5-hydroxy-methyl-1-[(2'-1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole; EXP3174, (2-n-butyl-4-chloro-1-[(2'-1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid); candesartan, (2ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-yl)methyl]-1H-benzimidazole-7-(carboxylic acid) in ATF}. Each tubule receiving the antagonist solution was exposed to only one concentration.

Statistics

In each experiment a mean value for J_{v_a} was determined from both control and treatment measurements. A paired *t*-test was performed for each group (different concentration of AT₁ antagonists) and compared with the corresponding controls. Values are expressed as means $\pm$ s.e.mean (n = number of rats).

Results

The first series of experiments were designed to determine the effects of losartan on fluid uptake. Losartan (10^{-8} M) when added to the intratubular solution decreased mean proximal fluid uptake by 17.5% (control 2.24 ± 0.06 , treatment $1.87 \pm 0.16 \times 10^{-4}$ mm³ mm⁻² s⁻¹; $n=8$, $P<0.05$). However, no effect of losartan on fluid uptake was apparent when applied at 10^{-7} M (control 2.54 ± 0.46 , treatment $2.38 \pm 0.17 \times 10^{-4}$ mm³ mm⁻² s⁻¹; $n=7$).

When EXP3174 (10^{-5} – 10^{-9} M) was added to the intratubular fluid a dose-dependent decrease in fluid absorption was observed. EXP3174 decreased fluid absorption by 38.3% at 10^{-5} M (all values in $\times 10^{-4}$ mm³ mm⁻² s⁻¹; control 2.30 ± 0.05 , treatment 1.42 ± 0.14 ; $n=6$, $P<0.05$), 41.0% at 10^{-6} M (control 2.31 ± 0.12 , treatment 1.36 ± 0.24 ; $n=7$, $P<0.01$), 32.4% at 10^{-7} M (control 2.48 ± 0.11 , treatment 1.68 ± 0.10 ; $n=6$, $P<0.01$), 23.5% at 10^{-8} M (control 2.15 ± 0.13 , treatment 1.64 ± 0.18 ; $n=8$, $P<0.05$) and 17.8% at 10^{-9} M (control 2.19 ± 0.08 , treatment 1.8 ± 0.11 ; $n=6$, $P<0.05$). EXP3174 at 10^{-10} M had no significant effect on fluid absorption (control 2.08 ± 0.11 , treatment 1.96 ± 0.29 ; $n=8$).

Addition of candesartan at 10^{-5} M to the intratubular fluid caused a decrease in fluid uptake by 23.6% (control 2.53 ± 0.15 , treatment $1.93 \pm 0.14 \times 10^{-4}$ mm³ mm⁻² s⁻¹; $n=6$, $P<0.05$). A similar decrease in absorption was also evident at 10^{-8} M, (control 2.47 ± 0.04 , treatment $1.93 \pm 0.12 \times 10^{-4}$ mm³ mm⁻² s⁻¹; $n=6$, $P<0.01$).

There was no significant difference between the levels of inhibition of fluid absorption with the highest concentration (10^{-5} M) of candesartan and EXP3174.

Figure 1 The effect of intratubular AT₁ antagonists on proximal tubular fluid absorption. Data are shown as means $\pm$ s.e.mean and presented as percentage changes in fluid absorption compared with control. Figures in parentheses indicate numbers of animals. * $P<0.05$, ** $P<0.01$ (paired *t*-test).

Discussion

The use of several AT₁ receptor antagonists in this study clearly indicates that endogenous luminal angiotensin II acts on apical AT₁ receptors to increase fluid uptake. The addition of an AT₁ antagonist to the tubule lumen would be expected to result in an increase in fluid absorption if a high (inhibitory) endogenous concentration of angiotensin II was present, but a decrease in fluid absorption if a low (stimulatory) endogenous concentration of angiotensin II existed. The results of the present study, where the luminal addition of losartan (10^{-8} M), EXP3174 (10^{-9} – 10^{-5} M) and candesartan (10^{-8} and 10^{-5} M) all decreased fluid absorption, support the latter contention. This strongly suggests that, at least in the Inactin anaesthetized rat, the endogenous concentration of angiotensin II in the lumen is within the range associated with stimulation of sodium transport and most likely to be between 10^{-12} – 10^{-10} M.

Losartan at 10^{-8} M decreased fluid absorption by 17.5%, supporting the previous finding by Quan & Baum (1996) who perfused rat proximal tubules with losartan (10^{-8} M) and observed a 35% decrease in fluid uptake. Interestingly, this group also observed a 40% decrease in fluid uptake during luminal infusion of enalaprilat (10^{-4} M). The known effects of intravenous losartan on renal function in anaesthetized rats also support the observed decrease in fluid absorption with luminal addition of losartan at 10^{-8} M. Xie *et al.* (1990) observed that intravenous losartan had a powerful inhibitory effect on bicarbonate and fluid absorption, and Zhuo *et al.* (1992) reported that it caused a 9% decrease in fractional proximal fluid absorption. Studies performed in the rabbit aorta, revealed that losartan binds competitively to the AT₁ receptor, and importantly, exhibits no partial agonist effect (Chiu *et al.*, 1990).

This present study is the first to establish a dose response relation between the luminal concentration of EXP3174 and fluid absorption in the presence of endogenous luminal angiotensin II. The dose-dependent decrease in absorption induced by EXP3174 showed a maximum effect of 41% at

10^{-6} M. This inhibition of proximal fluid absorption is in accord with observations made in anaesthetized dogs following intravenous infusion of EXP3174 ($0.5 \mu\text{g kg}^{-1} \text{min}^{-1}$) (Tama-ki *et al.*, 1993). Fractional excretion of sodium and fractional proximal excretion of sodium were increased, indicating a decrease in sodium absorption in the proximal tubule. The pattern of response also concurs with observations from *in vitro* preparations of rabbit aorta where EXP3174 inhibited angiotensin II-stimulated contractile responses in a dose-dependent manner (Wong *et al.*, 1990).

Candesartan applied to the lumen at either a high (10^{-5} M) or low (10^{-8} M) concentration decreased fluid uptake to a similar extent. The lack of dose-dependence may represent saturation of the inhibitory action at a concentration of 10^{-8} M or greater. Candesartan is reported to be an insurmountable antagonist that may interact with the same or a similar site on the AT_1 -receptor as losartan (Ojima *et al.*, 1997).

All three antagonists, losartan, EXP3174 and candesartan at 10^{-8} M decreased fluid absorption by approximately 20%. At a higher concentration (10^{-5} M), both EXP3174 and candesartan decreased fluid absorption, in contrast with previous observations that losartan at this dose increases transepithelial sodium transport (Hiranyachattada & Harris, 1996; Leyssac *et al.*, 1997). The dose-dependent, biphasic effect of losartan is not consistent with selective blockade of luminal AT_1 receptors, and suggests that losartan at high concentrations ($>10^{-6}$ M) has a nonselective effect on sodium absorption. It is not possible to determine the site of this non-selective effect of losartan at concentrations greater than 10^{-6} M from the results of this present study.

Losartan is known to have different pharmacological properties than EXP3174 and candesartan. Firstly, it is a surmountable antagonist as opposed to both EXP3174 and

candesartan displaying insurmountable antagonism (Wong *et al.*, 1990; Ojima *et al.*, 1997). In humans losartan increases uric acid secretion (Burnier *et al.*, 1996) although in rats both losartan and EXP3174 both inhibit urate uptake (Edwards *et al.*, 1996).

While the evidence presented here points to a low endogenous concentration of luminal angiotensin II, the data from measurements of angiotensin II in fluid samples obtained directly from the proximal tubule lumen (Seikaly *et al.*, 1990; Braam *et al.*, 1993) cannot be easily dismissed. Measurements obtained from free-flow collection samples revealed nanomolar concentrations of angiotensin II, and may differ from the endogenous level in split-droplets because of angiotensin II filtered at the glomerulus. Although, inspection of the dose-response relations produced by luminal application of exogenous angiotensin II (Wang & Chan 1990; Li *et al.*, 1994), suggest that no significant effect of endogenous angiotensin II on fluid absorption would be observed if the concentration was around 10^{-9} M.

It is concluded from the pharmacological studies using AT_1 receptor antagonists that angiotensin II is present in the lumen at a concentration that stimulates sodium reabsorption (around 10^{-12} – 10^{-10} M). The use of appropriate concentrations of pharmacological antagonists indicated that losartan has a nonselective action on fluid absorption at concentrations greater than 10^{-6} M. Losartan at a low concentration (10^{-8} M) has a quantitatively similar action on fluid uptake compared with EXP3174 and candesartan at the same dose.

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