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EFFECTS OF RILMENIDINE ON PROXIMAL TUBULAR FLUID ABSORPTION IN RATS

Valentina Jovanovska, Eveline Eitle and Peter J Harris

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

SUMMARY

1. The antihypertensive agent rilmenidine has threefold higher affinity for I_1 imidazoline receptors compared with α_2-adrenoceptors and acts on the central nervous system by reducing sympathetic activity and in the kidney by inhibiting Na^+/H^+ exchange activity.

2. In the present study, we examined: (i) the effects of luminal and peritubular administration of rilmenidine on fluid absorption in superficial proximal tubules; and (ii) the nature of the receptors involved in mediating the action of this drug in the presence of specific antagonists (efaroxan, idazoxan and 2-methoxy-idazoxan). Studies were performed in anaesthetized Sprague-Dawley rats using shrinking split-drop micropuncture.

3. Luminal administration of rilmenidine (10^{-5} and 10^{-13} mol/L) inhibited proximal tubular fluid absorption. Peritubular rilmenidine at 10^{-12} and 10^{-13} mol/L also inhibited fluid uptake, whereas rilmenidine at 10^{-11} mol/L had a significant stimulatory action.

4. In the presence of the $I_2 > I_1/\alpha_2$-adrenoceptor antagonist idazoxan (10^{-5} mol/L), luminal rilmenidine (10^{-5} mol/L) stimulated fluid absorption. Stimulation of fluid uptake was also observed when rilmenidine (10^{-5} mol/L) and the I_1 imidazoline receptor antagonist efaroxan (10^{-5} mol/L) were added together in the luminal fluid. Luminal administration of the selective α_2-adrenoceptor antagonist 2-methoxy-idazoxan (10^{-5} mol/L) resulted in significant attenuation of the inhibitory action of luminal rilmenidine (10^{-5} mol/L). This indicates that both I_1 imidazoline receptors and α_2-adrenoceptors are involved in the luminal actions of rilmenidine.

5. The effects of luminal and peritubular administration of α-methylnoradrenaline (an α_2-adrenoceptor agonist) were compared with those of rilmenidine. Luminal α-methylnoradrenaline, at higher concentrations (10^{-7} and 10^{-5} mol/L), inhibited fluid absorption, as was seen with peritubular rilmenidine, but, in contrast with rilmenidine, no stimulatory action was observed. Peritubular α-methylnoradrenaline inhibited fluid uptake at higher concentrations (10^{-5} and 10^{-7} mol/L), whereas rilmenidine at these concentrations had no effect. The differences in the concentration-dependent responses for rilmenidine and α-methylnoradrenaline indicate that both imidazoline receptors and α_2-adrenoceptors are involved in the actions of these compounds on proximal fluid uptake.

Key words: α_2-adrenoceptors, α-methylnoradrenaline, efaroxan, fluid absorption, idazoxan, imidazoline (I_1) receptors, 2-methoxy-idaxozan, micropuncture, rat proximal tubules, rilmenidine.

INTRODUCTION

The antihypertensive effect of rilmenidine appears to involve both central and peripheral actions characterized by suppression of sympathetic nerve outflow. The central action has been proposed to involve the rostral ventrolateral medulla,[1] whereas the peripheral action occurs at the kidney and is due, at least partially, to a reduction of the sodium-retaining influence of the renal sympathetic nerves.[2,3] Other studies have suggested that rilmenidine acts directly on the proximal tubule to inhibit sodium reabsorption, thus inducing natriuresis and lowering blood pressure.[4] Proximal tubular sodium reabsorption is regulated by a mechanism that involves luminal Na^+/H^+ exchanger isoform 3 (NHE3) and it has been reported[5] that enhanced activity of this exchanger is associated with sodium retention during the early stages of the development of high blood pressure in the spontaneously hypertensive rat (SHR) model of essential hypertension.

Knowledge of the cellular actions of rilmenidine is essential to an understanding of the renal actions of this compound and it is likely that these mechanisms are similar to those involved in physiological control by peptides and sympathetic neurotransmitters.[6] Rilmenidine may act via α_2-adrenoceptors and there is evidence that this receptor is involved in mediating the stimulatory action of noradrenaline on proximal tubular sodium transport.[7,8] However, the results of studies evaluating the adrenoceptor subtype responsible for this action are controversial. In studies on anaesthetized rabbits Hesse and Johns[9,10] administered selective α_2-adrenoceptor agonists intrarenally at doses that did not change renal haemodynamics and found significant decreases in urine flow and in both absolute and fractional Na^+ excretion. The authors concluded that adrenergic stimulation of renal Na^+ and water reabsorption involves α_1- but not α_2-adrenoceptors. This report was confirmed by Osborn *et al*.,[11] whereas others have reported the involvement of α_2-adrenoceptors in this response.[12,13]

It is known that imidazoline and guanidinium α_2-adrenoceptor compounds are also recognized by imidazoline-preferring receptors (IPR) and these receptors appear to be involved in the regulation of proximal tubule reabsorption. Binding studies show the presence of both α_2-adrenoceptors and imidazoline receptors in rabbit proximal tubules.[14,15]

Correspondence: Professor Peter J Harris, Department of Physiology, Faculty of Medicine, Dentistry & Health Sciences, The University of Melbourne, Victoria 3010, Australia. Email: pjharris@unimelb.edu.au

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Rilmenidine has been reported to be an agonist on both IPR and α_2-adrenoceptors.[16,17] In whole-animal studies, Kline and Cechetto[2] observed that rilmenidine increased renal blood flow by a mechanism independent of renal nerves and increased sodium excretion indirectly by decreasing renal sympathetic nerve activity. From these experiments, it was not possible to determine whether there was a direct action of rilmenidine on proximal tubule cells.

Taken together, these studies leave open the question of the nature of the receptors mediating the effects of rilmenidine on proximal tubule sodium transport.

In the present study, we examined the effects on fluid absorption of rilmenidine administered locally into the luminal or peritubular fluids surrounding superficial proximal tubules. Selective pharmacological antagonists were then used to identify the involvement of imidazoline receptors. α-Methylnoradrenaline is a selective α_2-adrenoceptor agonist with very low affinity for I_1 receptors[18,19] and its actions were compared with those of rilmenidine, which may affect both imidazoline and α_2-adrenoceptors.

METHODS

Adult male Sprague-Dawley rats (Biological Research Facility, Departments of Physiology and Pharmacology, University of Melbourne) were anaesthetized with thiobutabarbital (110 mg/kg, i.p.) and infused intravenously with 0.9% NaCl at 1.6 mL/h per 100 g bodyweight. The right carotid artery was cannulated to monitor blood pressure, which was recorded on a chart recorder (Cardiotrace Physiological Recorder; Watson-Victor, Sydney, NSW, Australia). The left kidney was prepared for micropuncture and, following an equilibration period of 1–2 h, shrinking split-drop microperfusion performed in mid-proximal convoluted tubule segments visible on the kidney surface.[20,21]

Sudan Black-stained castor oil was first introduced into a proximal tubule from one barrel of a double-barrelled micropipette. An artificial tubular fluid solution (145 mmol/L NaCl, 5 mmol/L $NaHCO_3$, 5 mmol/L KCl and 1.5 mmol/L $CaCl_2$) was then injected from the other barrel to split the oil column. The rate of shrinking of the split-drop was determined by digital image analysis of the positions of the oil–water menisci in successive video frames captured at 1 s intervals. In each rat, proximal fluid uptake rate per unit surface area of epithelium (Jv_a) was determined in three to five tubules and a mean value calculated. Fluid absorption rate was then determined in a further three or more tubules using intratubular fluid containing rilmenidine with or without antagonists.

Perfusion of the peritubular capillaries surrounding the split-drop was performed to investigate the effect on proximal fluid uptake of peritubular delivery of rilmenidine or α-methylnoradrenaline.

Peritubular capillaries were perfused with a control solution similar to plasma, but without addition of protein, and introduced using gas (95% O_2/5% CO_2) applied to a micropipette at a pressure sufficient to clear the blood from all capillaries adjacent to the split-drop. Average values for fluid uptake rates were obtained in three to five tubules during peritubular perfusion with control fluid and then in a further three to five tubules during perfusion with a similar fluid containing rilmenidine or α-methylnoradrenaline. Solutions for luminal or peritubular perfusion were labelled by code before being handed to the micropuncturist ready for filling the pipettes. The order of administration of solutions was random.

Drugs

Rilmenidine was supplied by Servier (Paris, France). 2-Methoxy-idazoxan and efaroxan were obtained from Sigma–Aldrich (Sydney, NSW, Australia). Idazoxan and α-methylnoradrenaline were obtained from Research Biochemicals International (Sydney, NSW, Australia).

Statistical analysis

In each experiment, comparisons were made between the average values for fluid uptake rate (Jv_a) using control and test solutions during either luminal or peritubular capillary perfusion. Student's paired *t*-test was used to evaluate statistical significance. The significance level was designated as $P < 0.05$.

RESULTS

In the present study, dose–response relationships for the effects of luminal and peritubular additon of rilmenidine and α-methylnoradrenaline on proximal tubular fluid uptake were examined. The concentrations of the compounds ranged between 10^{-5} and 10^{-13} mol/L.

Rilmenidine (10^{-5} mol/L), when added to the lumen of proximal tubules, decreased fluid absorption by 17.3% ($n = 6$; $P < 0.05$; Fig. 1a; Table 1). A significant inhibitory effect of 10.9% was also

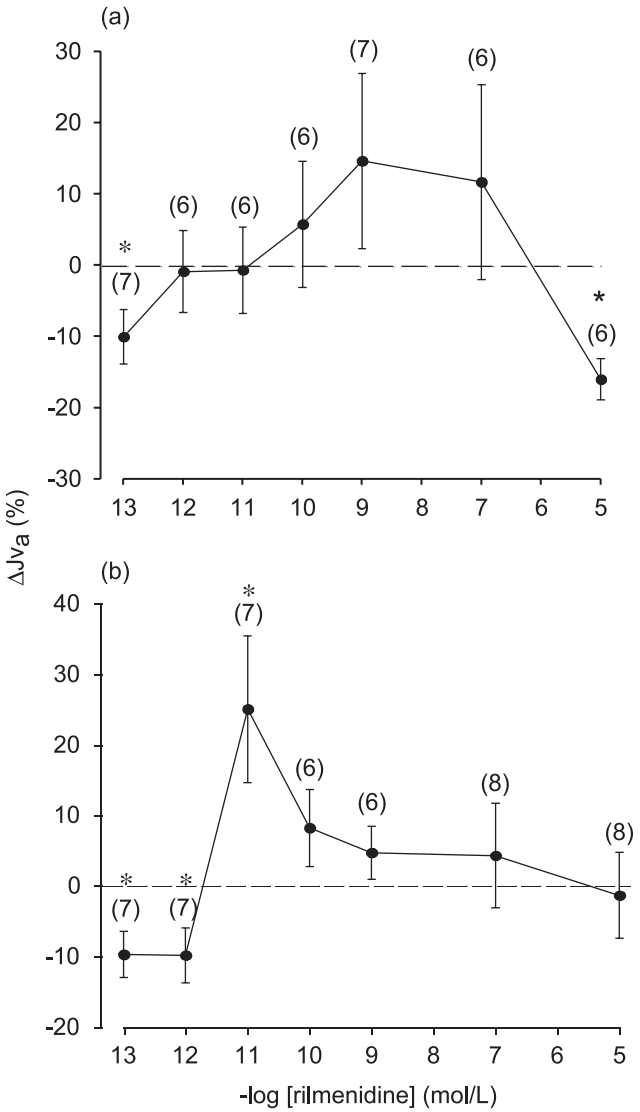

Fig. 1 Dose–response relationship for the percentage changes in proximal tubular fluid absorption rate (Jv_a) during (a) luminal and (b) peritubular administration of rilmenidine. Data are the mean±SEM. Numbers in parentheses indicate the number of animals. *$P < 0.05$ compared with control (paired *t*-test).

observed with rilmenidine at 10^{-13} mol/L ($n = 7$; $P < 0.05$; Fig. 1a; Table 1).

Rilmenidine at 10^{-7}, 10^{-9}, 10^{-10}, 10^{-11} and 10^{-12} mol/L had no significant effect on proximal fluid uptake rate. Thus, only the highest (10^{-5} mol/L) and lowest (10^{-13} mol/L) doses of rilmenidine were found to be effective in inhibiting proximal tubular sodium and water reabsorption *in vivo*. The effects of luminal administration of rilmenidine on proximal tubular fluid uptake are shown in Fig. 1a.

Peritubular administration of rilmenidine at 10^{-5}, 10^{-7}, 10^{-9} and 10^{-10} mol/L did not affect the proximal fluid uptake rate (Fig. 1b, Table 1). However, at a lower concentration, peritubular rilmenidine (10^{-11} mol/L) increased mean proximal fluid uptake by 21.4% ($n = 7$; $P < 0.05$; Fig. 1b; Table 1). At 10^{-12} mol/L, the effect was opposite, showing inhibition of fluid uptake by 10.2% ($n = 7$; $P < 0.05$; Fig. 1b; Table 1). Similarly, at 10^{-13} mol/L, rilmenidine inhibited fluid absorption by 9.6% ($n = 7$; $P < 0.05$; Fig. 1b; Table 1).

Antagonists with different affinities for I_1 imidazoline and α_2-adrenoceptors were used as a comparison with the agonist rilmenidine.

In the presence of the α_2-adrenoceptor antagonist 2-methoxy-idazoxan (10^{-5} mol/L), luminal rilmenidine (10^{-5} mol/L) attenuated the inhibitory action of rilmenidine (Table 2).

However, administration of rilmenidine (10^{-5}M) with the antagonist idazoxan (10^{-5} mol/L; affinity I_2 receptors > α_2-adrenoceptors) caused an increase in the fluid absorption rate of 19.3% ($n = 7$; $P < 0.05$; Table 2). An increase in the fluid uptake rate of 15.6% was also observed when rilmenidine (10^{-5} mol/L) was applied to the lumen of proximal tubules together with the selective imidazoline I_1 receptor antagonist efaroxan (10^{-5} mol/L; $n = 8$; $P < 0.05$; Table 2). Therefore, the antagonists idazoxan and efaroxan were much more potent in attenuating the actions of rilmenidine in proximal tubules then 2-methoxy-idazoxan.

We also determined whether the antagonists alone had any direct effects on the proximal tubule fluid absorption rate at the doses used in the present studies.

Luminal administration of 2-methoxy-idazoxan (10^{-5} mol/L) had no effect on fluid absorption and, similarly, no effect was observed with idazoxan (10^{-5} mol/L) and efaroxan alone (Table 2).

We also investigated whether the effects of α-methylnoradrenaline (a selective α_2-adrenoceptor agonist) on proximal tubule fluid absorption differed from those of rilmenidine.

Luminal administration of α-methylnoradrenaline (10^{-5} mol/L) inhibited fluid uptake by 15.9% ($n = 6$; $P < 0.05$; Fig. 2a; Table 3). Inhibition of the proximal fluid absorption rate was also evident at 10^{-7} mol/L α-methylnoradrenaline (8.2%; $n = 6$; $P < 0.05$; Fig. 2a; Table 3), whereas at lower concentrations (10^{-9}, 10^{-11}, 10^{-12} and 10^{-13} mol/L) α-methylnoradrenaline had no effect (Fig. 2a).

The dose–response relationship for the peritubular administration of α-methylnoradrenaline reveals inhibition of fluid uptake at 10^{-5}, 10^{-7}, 10^{-12} and 10^{-13}mol/L (18.2, 16.7, 6.9 and

Table 1 Mean proximal tubular fluid absorption rate following the luminal addition or peritubular capillary perfusion of rilmenidine

	Jv_a ($\times 10^{-4}$ mm^3/mm^2 per s)					
	Luminal perfusion			Peritubular perfusion		
	n	Control	Rilmenidine	*n*	Control	Rilmenidine
Concentration of rilmenidine (mol/L)						
10^{-5}	6	3.18 ± 0.22	2.63 ± 0.48*	8	2.11 ± 0.11	2.05 ± 0.07
10^{-7}	6	2.48 ± 0.17	2.72 ± 0.29	8	2.13 ± 0.11	2.19 ± 0.14
10^{-9}	7	2.72 ± 0.11	3.04 ± 0.19	6	2.34 ± 0.04	2.45 ± 0.09
10^{-10}	6	2.60 ± 0.19	2.67 ± 0.10	6	2.20 ± 0.11	2.36 ± 0.10
10^{-11}	6	2.64 ± 0.16	2.60 ± 0.15	7	1.92 ± 0.12	2.33 ± 0.08*
10^{-12}	6	2.42 ± 0.07	2.38 ± 0.10	7	2.44 ± 0.10	2.19 ± 0.11*
10^{-13}	7	2.93 ± 0.14	2.61 ± 0.08*	7	2.61 ± 0.06	2.36 ± 0.10*

Data are the mean±SEM (*n* = number of animals). *$P < 0.05$ compared with control fluid (paired *t*-test).
Jv_a, proximal tubular fluid absorption rate.

Table 2 Mean values of fluid absorption rate following the administation of rilmenidine and an antagonist together or the antagonist alone into the lumen of proximal tubules and control tubular fluid

	n	Jv_a ($\times 10^{-4}$ mm^3/mm^2 per s)
Luminal perfusion		
Control	6	2.93 ± 0.15
Rilmenidine 10^{-5} mol/L + 2-MI 10^{-5} mol/L		2.65 ± 0.13
Control	7	2.85 ± 0.14
Rilmenidine 10^{-5} mol/L + idazoxan 10^{-5} mol/L		3.40 ± 0.05*
Control	8	2.57 ± 0.07
Rilmenidine 10^{-5} mol/L + efaroxan 10^{-5} mol/L		2.97 ± 0.08*
Control	5	3.06 ± 0.19
2-MI 10^{-5} mol/L		3.08 ± 0.12
Control	6	2.99 ± 0.10
Idazoxan 10^{-5} mol/L		2.85 ± 0.08
Control	8	2.75 ± 0.04
Efaroxan 10^{-5} mol/L		2.78 ± 0.10

Data are the mean±SEM (*n* = number of animals). *$P < 0.05$ compared with control fluid (paired *t*-test).
Jv_a, proximal tubular fluid absorption rate; 2-MI, 2-methoxy-idazoxan.

14.9%, respectively; $n = 5$ in each group; $P < 0.05$), whereas α-methylnoradrenaline at concentrations of 10^{-9} and 10^{-11}mol/L had no significant effect on fluid absorption rate (Fig. 2b; Table 3).

DISCUSSION

Many studies support the hypothesis that the activation of imidazoline receptors leads to natriuresis through inhibition of Na^+/H^+ exchange in renal tubules.[4,22] It has been suggested that I_1 receptors are important in mediating the hypotensive actions of clonidine, moxonidine and rilmenidine and may be involved in the regulation of renal sodium and water excretion.[22,23] It has also been reported that α_2-adrenoceptors can modulate sodium reabsorption in the proximal tubule[13] and there is convincing evidence that α_2-adrenoceptor agonists have direct effects on fluid reabsorption in the kidney through actions on the Na^+/H^+ exchanger in the proximal tubule.[8,24] Bidet *et al*.[4] demonstrated that imidazoline derivatives modulate Na^+ influx independently of the interaction with α_2-adrenoceptors.

We designed a series of experiments to investigate the direct effects of rilmenidine and α-methylnoradrenaline on proximal fluid absorption and to characterize the receptors by which they may exert their effects.

In the present study, luminal administration of the I_1 receptor agonist rilmenidine (10^{-5} mol/L) inhibited transepithelial absorption of sodium and water reabsorption in proximal tubules. This effect is consistent with the previously reported effects of rilmenidine (10^{-5} mol/L) on [^{22}Na] uptake in isolated proximal cells.[4] This inhibitory effect of rilmenidine was reversed by the selective I_2 receptor/α_2-adrenoceptor antagonist idazoxan, which has been reported to have higher affinity for I_2 receptors compared with I_1 receptors[17,25] and the I_1 receptor/α_2-adrenoceptor antagonist efaroxan. In contrast, the highest dose of the α_2-adrenoceptor antagonist 2-methoxy-idazoxan significantly attenuated the inhibitory action of rilmenidine.

Previous studies by Allan *et al*.[22] similarly showed that the renal actions of moxonidine, an I_1 receptor agonist were blocked by idazoxan, but not by the selective α_2-adrenoceptor antagonist rauwolscine. Schlatter *et al*.[26] found that the inhibition of NHE activity produced by moxonidine was not affected by the α_2-adrenoceptor antagonist yohimbine, which is in contrast with our data showing that there was effect of 2-methoxy-idazoxan on rilmenidine-induced inhibition of fluid uptake added into the lumen of proximal tubules.

However, it is surprising that an I_2 receptor-selective antagonist (idazoxan) is effective in blocking the inhibitory actions of an I_1 receptor-specific agonist (rilmenidine). The affinity of efaroxan for

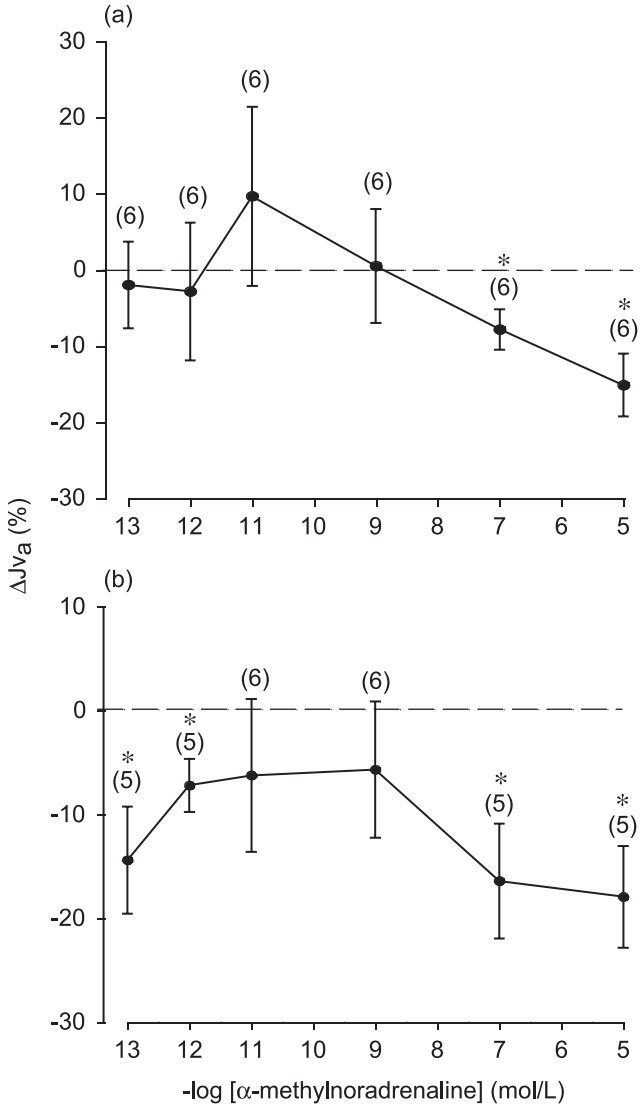

Fig. 2 Dose–response relationship for the percentage changes in proximal tubular fluid absorption rate (Jv_a) during (a) luminal and (b) peritubular administration of α-methylnoradrenaline. Data are the mean±SEM. Numbers in parentheses indicate the number of animals. *$P < 0.05$ compared with control (paired *t*-test).

Table 3 Mean values of proximal tubular fluid absorption rate following luminal or peritubular administration of α-methylnoradrenaline

	Jv_a ($\times 10^{-4}$ mm^3/mm^2 per s)					
	Luminal perfusion			Peritubular perfusion		
	n	Control	α-MNA	*n*	Control	α-MNA
Concentration of α-MNA (mol/L)						
10^{-5}	6	2.76 ± 0.14	2.32 ± 0.05*	5	2.47 ± 0.07	2.02 ± 0.10*
10^{-7}	6	2.70 ± 0.22	2.48 ± 0.16*	5	2.51 ± 0.06	2.09 ± 0.13*
10^{-9}	6	2.96 ± 0.10	2.95 ± 0.15	6	2.42 ± 0.10	2.26 ± 0.14
10^{-11}	6	2.38 ± 0.19	2.54 ± 0.20	6	2.14 ± 0.13	1.97 ± 0.12
10^{-12}	6	2.41 ± 0.12	2.30 ± 0.14	5	2.32 ± 0.14	2.16 ± 0.15*
10^{-13}	6	2.71 ± 0.18	2.66 ± 0.18	5	2.41 ± 0.16	2.05 ± 0.15*

Data are the mean±SEM (*n* = number of animals). *$P < 0.05$ compared with control fluid (paired *t*-test).
Jv_a, proximal tubular fluid absorption rate; α-MNA, α-methylnoradrenaline.

I_1 receptors is much higher than its affinity for α_2-adrenoceptors. Ernsberger *et al*.[17] reported that efaroxan was highly specific for the I_1 site over the I_2 site. In our studies, we observed that the effects of luminal rilmenidine were antagonized by the I_1 receptor ligand efaroxan rather than the more selective α_2-adrenoceptor antagonist 2-methoxy-idazoxan, indicating that rilmenidine may promote sodium excretion through an interaction with I_1 receptors rather than α_2-adrenoceptors.

The studies using efaroxan as a selective antagonist for rilmenidine suggest either the involvement of two different receptors or the existence of two processes coupled to one receptor (one mediating stimulation and the other inhibition) modulating sodium absorption in proximal tubules.

Sodium and water uptake were inhibited by lower concentrations (10^{-12} and 10^{-13} mol/L) of rilmenidine when administered to the peritubular side. A similar effect was observed with luminal administration of rilmenidine at 10^{-13} mol/L. Interestingly, at a higher concentration (10^{-11} mol/L) rilmenidine had a stimulatory action, indicating the presence of a peritubular imidazoline-preferring receptor. It is important to note that there are some specific points in the dose–response studies for rilmenidine that are crucial in reaching conclusions concerning the biphasic pattern of stimulation and inhibition of fluid transport. In particular, we report here that low concentrations of rilmenidine (10^{-13} and 10^{-12} mol/L) inhibit transport, whereas a 10-fold increase in concentration (10^{-11} mol/L) results in reversal of the effect and marked stimulation of fluid uptake. Possible explanations for such a biphasic, dose-dependent action involve interactions between rilmenidine and endogenous agonist systems, such as angiotensin, endothelin, vasopresin, nitric oxide or catecholamines, or, alternatively, activation of multiple receptors or multiple intracellular signalling pathways.

The involvement of α_2-adrenoceptors in the responses to rilmenidine were further examined by comparing these responses with those observed in response to the α_2-adrenoceptor agonist α-methylnoradrenaline. Previous studies by Gesek and Schoolwerth[27] showed that α_2-adrenergic agonists stimulate Na^+/H^+ exchange in proximal tubule cells from SHR and normotensive Wistar-Kyoto rats. In proximal tubular cells, α_1 and α_2-adrenoceptors exert synergistic effects on Na^+/H^+ exchange, enhancing sodium uptake.[24,28] In our experimental model, the dose–response relationship of peritubular α-methylnoradrenaline indicated inhibition of Na^+/H^+ exchange at higher and lower concentrations, supporting the involvement of α_2-adrenoceptors in inhibiting tubular sodium reabsorption. These data are in contrast with previously published studies showing that stimulation of α_2-adrenoceptors localized on the basolateral side of rat, rabbit and human renal proximal tubules increases tubular sodium reabsorption.[29–31]

Lower concentrations (10^{-12} and 10^{-13} mol/L) of peritubular α-methylnoradrenaline inhibited fluid uptake, similar to the responses we obtained with rilmenidine. It appears likely that the same receptors are involved in the actions of both drugs. However, we found important differences between these two compounds at higher concentrations.

Luminal and peritubular α-methylnordarenaline showed inhibitory actions at 10^{-5} and 10^{-7} mol/L, whereas an inhibitory action of luminal rilmenidine was observed only at 10^{-5} mol/L. Because rilmenidine has a relatively high selectivity for I_1 receptors and α-methylnordarenaline is a selective agonist for the α_2-adrenoceptors that has negligible affinity for the I_1 receptors, it appears that this action is mediated through two distinct receptors, one an imidazoline receptor and the other α_2-adrenoceptors.

Therefore, our data suggests the involvement of both imidazoline and α_2-adrenoceptors in mediating inhibition of fluid transport in proximal tubules.

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