Attenuated renal response to moxonidine and rilmenidine in one kidney-one clip hypertensive rats

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Introduction

In acquired forms of hypertension, renal α-adrenoceptor density has been found to be unchanged or decreased (Yamada et al., 1980; Fukuda et al., 1983; Saiz et al., 1987; Michel et al., 1989b; Wilson, 1991), whereas in spontaneously hypertensive rats, the density of renal α-adrenoceptors has been found to be elevated as compared to Wistar-Kyoto rats (Pettinger et al., 1982; Saiz et al., 1987; Sanchez et al., 1989; Michel et al., 1990a). Furthermore, previous studies in our laboratory indicated that the renal response to α1-adrenoceptor agonists such as clonidine was decreased in spontaneously hypertensive rats (Li et al., 1992) but not in one kidney, one clip (1K-1C) hypertensive rats (Li & Smyth, 1994). The diuretic and natriuretic effects of α1-adrenoceptor stimulation have been shown to be mediated by an inhibition of the renal action of vasopressin (Blandford & Smyth, 1990; Gellai, 1990). Thus, the decreased renal response to a V2-vasopressin receptor antagonist in spontaneously hypertensive rats but not 1K-1C hypertensive rats (Li & Smyth, 1993) was consistent with the decreased response to α1-adrenoceptor stimulation in this model of genetic hypertension.

Recent studies in our laboratory have proposed that the effects of two purported α2-adrenoceptor agonists, clonidine and 2,6-dimethyl clonidine (2,6-DMC), may in fact be mediated by two α2-adrenoceptor subtypes or two distinct receptors, α2A-adrenoceptors and non-adrenoceptor, imidazoline receptors (Smyth et al., 1992a). Whereas 2,6-DMC caused an increase in urine flow rate which was secondary to an increase in osmolar clearance, clonidine resulted in an increase in urine flow rate which was secondary to an increase in free water clearance (Smyth et al., 1992a). Furthermore, we have recently found that the effects of 2,6-DMC but not clonidine were significantly attenuated in rats with 1K-1C hypertension (Li & Smyth, 1994) and there was a decrease in [3H]-idazoxan binding sites in kidneys from 1K-1C hypertensive rats. The renal actions of 2,6-DMC can be antagonized by idazoxan (Smyth & Li, 1991) which would be consistent with those effects being mediated by the non-adrenoceptor, imidazoline receptor (Feldman et al., 1990; Gomez et al., 1991; Allan et al., 1993). Nevertheless, it has not been determined as to whether the decreased response to 2,6-DMC was due to a decreased activity at the α2-adrenoceptor or the non-adrenoceptor, imidazoline receptor.

In the present study, we determined whether or not, in 1K-1C hypertensive rats, a decreased response was found with non-adrenoceptor, imidazoline receptor agonists in general. Two agonists (rilmenidine and moxonidine) which have been shown to display a much greater selectivity and affinity for the α1, non-adrenoceptor, imidazoline receptor over the α2-adrenoceptor (Ernsberger et al., 1992), were used to investigate further the non-adrenoceptor, imidazoline receptor in 1K-1C hypertensive and 1K-1C normotensive rats.

Methods

Experimental animals

The standard procedures have been described previously (Blandford & Smyth, 1988; 1990). Male Sprague-Dawley rats (100–125 g) were obtained from the University of Manitoba (Charles River Breeding Stock) and cared for according to regional animal care standards protocol. Animals were housed at 22°C with an environmental humidity maintained at 50% with a light/dark cycle from 07 h 00 min to 19 h 00 min. Rats were fed Purina rat chow and received tap water for drinking.

The animals were separated into two groups. In the first group (1K-1C), rats were anaesthetized with ether and the kidneys exposed by an abdominal incision. A silver clip

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Renal effects of rilmenidine or moxonidine

On the day of the experiment, the rats were anaesthetized with pentobarbitone (BDH Chemicals, Poole, England; 50 mg kg\(^{-1}\), i.p.). Additional anaesthetic was administered in a bolus dose of 3 mg kg\(^{-1}\) i.v. as needed. The rats were placed on a thermostatically controlled heating blanket. The rectal thermometer was connected to a Harvard Animal Blanket Control Unit which was used to maintain body temperature at 37.5°C. The trachea was cannulated with polyethylene tubing (PE-240) and the animal was allowed to breathe spontaneously. The left carotid artery was cannulated with polyethylene tubing (PE-50) and blood pressure was measured with a Statham pressure transducer (model P23DC) connected to a Grass Polygraph Model V. The left jugular vein was cannulated (PE-160) and connected to an infusion pump (Syringe pump model 355) for the infusion of saline. The left kidney was exposed by a flank incision and the left ureter was cannulated (PE-50) for the collection of urine. A 30 gauge stainless steel needle was inserted into the aorta and advanced into the origin of the left renal artery. A single drop of glue (Superglue, LePage's Limited) was used to secure the base of the needle to surrounding tissue. This was connected by Tygon Tubing (size 0.25 mm) to an infusion pump (Harvard infusion/withdrawal pump model 600–900) for infusion of either saline or study drug at 3.4 μl min\(^{-1}\).

Following the surgical procedure, saline was administered by intravenous infusion (97 μl min\(^{-1}\)). The animals were allowed to stabilize for a 45 min period. After the stabilization period, a 15 min control urine collection was obtained. This was followed by infusion of saline (vehicle), rilmenidine (3, 10 or 30 nmol kg\(^{-1}\) min\(^{-1}\)) or moxonidine (1, 3 or 10 nmol kg\(^{-1}\) min\(^{-1}\)) directly into the renal artery for the duration of the experiment at 3.4 μl min\(^{-1}\). During this infusion, two additional urine collections of 15 min and 45 min were obtained into preweighed tubes.

The sodium concentration in urine and plasma were determined with a Beckman KLiNa Flame Photometer. Creatinine concentration was determined with a Beckman Creatinine Analyzer Model 2. The urine and plasma osmolality was analysed with a MicroOsmette (Precision Systems). Urine volume was determined gravimetrically.

**Data analysis**

All data are presented as the mean ± the standard error of the mean (s.e.mean). Statistical analysis was performed with repeated-measures ANOVA using SAS System Version 6.07. Significant interactions were further analyzed with Least Squares Means Difference Test. Baseline values (first urine collection) prior to the administration of the saline vehicle or study drugs (moxonidine and rilmenidine) were compared and presented in table format. The effects of the saline vehicle and study drugs were expressed graphically as the absolute change from the first to final urine collection period. This allowed the determination of the magnitude of the changes for each variable within the different groups. Each group comprised 5 to 8 animals. In the figures, *denotes P<0.05 between groups (1K vs 1K-IC) receiving the same drug infusion rate and #denotes P<0.05 as compared with the control within the same group receiving the vehicle infusion.

**Drugs**

Moxonidine (supplied by Beiersdorf, AG, Hamburg, Germany) and rilmenidine (supplied by I.R.I. Servier, France) were used.

**Results**

Effect of intrarenal infusion of rilmenidine

Baseline values for experimental groups prior to the administration of the experimental treatments (vehicle, rilmenidine) are shown in Table 1. The first collection period was used as an indication of the effects of the surgical preparation in the different groups. The baseline values were similar for all the different doses within either the 1K sham or 1K-1C hypertensive groups. The 1K-1C hypertensive rats consistently had higher blood pressures (P<0.05) than the 1K sham animals. The incidence of hypertension in the animals which received the renal clip was 100%.

The mean arterial blood pressure was not altered by rilmenidine in either the 1K-sham normotensive rats or the 1K-1C hypertensive rats (Figure 1). Heart rate was decreased

| Table 1 Absolute baseline values for experimental groups prior to the administration of vehicle (0 nmol kg\(^{-1}\) min\(^{-1}\)) or rilmenidine (3, 10 and 30 nmol kg\(^{-1}\) min\(^{-1}\)) |
|-----------------------------------|-----------------------------------|-----------------------------------|
| **1K-Sham**                       | **Rilmenidine**                   | **1K-IC**                         |
| **BP (mmHg)**                     | 0                                | 0                                | 10 | 30 |
| **HR (b.p.m.)**                   | 121 ± 2                          | 0                                | 165 ± 4* | 168 ± 7* |
| **C\(_{cr}\) (ml min\(^{-1}\))**  | 433 ± 10                         | 150 ± 10                         | 147 ± 15 | 437 ± 13 |
| **UV (μl min\(^{-1}\))**          | 1.6 ± 0.3                        | 0                                | 417 ± 15 | 463 ± 6 |
| **UNaV (μEq min\(^{-1}\))**       | 14 ± 2                           | 152 ± 19                        | 447 ± 8 | 420 ± 19 |
| **C\(_{Na}\) (μl min\(^{-1}\))**  | 1.8 ± 0.2                        | 1.5 ± 0.1                       | 417 ± 8 | 402 ± 19 |
| **C\(_{Na}\) (μl min\(^{-1}\))**  | 17 ± 3                           | 1.5 ± 0.1                       | 447 ± 8 | 420 ± 19 |
| **C\(_{Na}\) (μl min\(^{-1}\))**  | 13 ± 1                           | 1.5 ± 0.1                       | 447 ± 8 | 420 ± 19 |
| **C\(_{Na}\) (μl min\(^{-1}\))**  | 14 ± 2                           | 152 ± 19                        | 447 ± 8 | 420 ± 19 |
| **BP, blood pressure; HR, heart rate; C\(_{cr}\), creatinine clearance; UV, urine flow rate; UNaV, sodium excretion; C\(_{Na}\), osmolar clearance; C\(_{Na}\), free water clearance. Values represent mean ± standard error. * denotes P<0.05 between 1K-sham normotensives and 1K-1C hypertensive rats.** |
only in 1K-1C rats at the highest infusion rate of rilmenidine (30 nmol kg⁻¹ min⁻¹). Rilmenidine did not alter creatinine clearance. All doses of rilmenidine resulted in an increase in urine flow rate and sodium excretion in 1K-sham rats (Figure 2). However, in 1K-1C hypertensive rats, no change in urine flow rate or sodium excretion was observed (Figure 2). This was reflected in significantly greater sodium excretions and urine flow rates for all doses in the 1K-sham animals. Rilmenidine increased osmolar clearance in 1K-sham normotensive rats but failed to have any effect in 1K-1C hypertensive rats (Figure 3). Free water clearance was not significantly altered in 1K-sham rats or 1K-1C hypertensive rats (Figure 3).

**Effect of intrarenal infusion of moxonidine**

Baseline values for experimental groups prior to the administration of the experimental treatments (vehicle, moxonidine) are shown in Table 2. The first collection period was used as an indication of the effects of the surgical preparation in the different groups. As would be anticipated, a significant interaction effect by group (1K Sham versus 1K-IC) was observed for blood pressure in that the animals which had received renal clips had a significantly higher blood pressure (P<0.05). Within the 1K sham rats the baseline values for heart rate (HR), osmolar clearance (CОс) and free water clearance (Ch0) in the animals that received 3 nmol kg⁻¹ min⁻¹ of moxonidine were different from the group which received the saline vehicle (Table 2). The differences were, a lower heart rate, increased osmolar clearance and decreased free water clearance (Table 2).

The mean arterial blood pressure was not altered by moxonidine in either the 1K-sham normotensive rats or the 1K-1C hypertensive rats (Figure 4). Heart rate was decreased in both 1K-sham and 1K-1C rats at the highest infusion rate of moxonidine (10 nmol kg⁻¹ min⁻¹). Moxonidine did not alter creatinine clearance. All doses of moxonidine resulted in an increase in urine flow rate in 1K-sham rats (Figure 5), whereas, in 1K-1C hypertensive rats, the two highest infusion rates increased urine flow rate (Figure 5). The increase in urine flow rate was also greater in the 1K-sham rats as compared to the 1K-1C rats. Sodium excretion was also increased by moxonidine in 1K-sham normotensive rats at all infusion rates investigated, but only at the highest infusion rate in 1K-1C hypertensive rats (Figure 5). Moxonidine increased osmolar clearance at all infusion rates investigated in 1K-sham normotensive rats but only at the highest dose in 1K-1C hypertensive rats (Figure 6). Free water clearance was increased in the 1K-sham rats at the lowest and highest doses of moxonidine but not the 1K-1C hypertensive rats.

![Figure 1](image1.png)  
**Figure 1** The effects of rilmenidine on mean arterial blood pressure, heart rate and creatinine clearance. Data are presented as means ± standard error of the mean. The solid columns represent the 1K-sham rats and the hatched columns represent the 1K-1C hypertensive rats. # denotes P<0.05 as compared with the respective control group.

![Figure 2](image2.png)  
**Figure 2** The effects of rilmenidine on urine flow rate and sodium excretion. The solid columns represent the 1K-sham rats and the hatched columns represent the 1K-1C hypertensive rats. * denotes P<0.05 between 1K-sham normotensive and 1K-1C hypertensive rats. # P<0.05 as compared with the respective control group.
Table 2 Absolute baseline values for experimental groups prior to the administration of vehicle (0 nmol kg⁻¹ min⁻¹) or moxonidine (1, 3 and 10 nmol kg⁻¹ min⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>1K-Sham</th>
<th>Moxonidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>121 ± 2</td>
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</tr>
<tr>
<td>HR (b.p.m.)</td>
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</tr>
<tr>
<td>C₃₆(μl min⁻¹)</td>
<td>1.6 ± 0.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>UV (μl/min)</td>
<td>14 ± 2</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>UNaV (μEq min⁻¹)</td>
<td>2.0 ± 0.4</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>C₈₀(μl/min)</td>
<td>53 ± 4</td>
<td>65 ± 6</td>
</tr>
<tr>
<td>CRH (μl/min)</td>
<td>-39 ± 4</td>
<td>-50 ± 4</td>
</tr>
</tbody>
</table>

BP, blood pressure; HR, heart rate; C₃₆, creatinine clearance; UV, urine flow rate; UNaV, sodium excretion; C₈₀, osmolar clearance; CRH, free water clearance. Values represent mean ± standard error. * denotes P < 0.05 between 1K-sham normotensives and 1K-IC hypertensive rats and *denotes P < 0.05 versus the vehicle control group (0) within the 1K-sham or 1K-IC groups.

Discussion

Boyajian et al. (1987) and Coupry et al. (1987) reported that two purported α₂-adrenoceptor antagonists, rauwolscine and idazoxan, labelled two distinct populations of receptors. Michel et al. (1989a; 1990b) also found that [³H]-idazoxan labelled a non-adrenoceptor site which was distinct from the α₂-adrenoceptor. These non-adrenoceptor sites also had a greater affinity for imidazoline based compounds than phenethylamines (catecholamines) (Bricca et al., 1989; Ernsberger et al., 1990). Recently these non-adrenoceptor, imidazoline sites have been found to exist as at least two subtypes, the I₁ which was labelled by [³H]-clonidine and the I₂ which was labelled by [³H]-idazoxan (Ernsberger et al., 1992; Reis et al., 1992).

A number of lines of evidence suggested that 2,6-DMC may be a non-adrenoceptor, imidazoline receptor agonist. Previous studies in our laboratory indicated that the two purported α₂-adrenoceptor agonists 2,6-DMC and clonidine increased urine flow rate by two different mechanisms (Smyth et al., 1992a). 2,6-DMC increased urine flow rate secondary to an increase in osmolar clearance while clonidine increased urine flow rate by increasing free water clearance. In addition, pretreatment with a V₂ vasopressin antagonist had no effect on the response to 2,6-DMC but completely attenuated the effects of clonidine. These results are consistent with the effects of 2,6-DMC and clonidine being mediated by two distinct mechanisms and/or sites, α₂-adrenoceptors and non-adrenoceptor, imidazoline receptors (Smyth et al., 1992a). Effects similar to those reported with 2,6-DMC have been found by Allan et al. (1993) using moxonidine, an α₂-adrenoceptor, imidazoline receptor agonist with an approximately 700 times higher affinity for I₁ non-adrenoceptor, imidazoline receptors than that for α₂-adrenoceptors in the kidney (Ernsberger et al., 1993). They found that moxonidine produced an increased urine flow rate in Sprague-Dawley rats and this increase was secondary to an increase in osmolar clearance. This increase was blocked by pretreatment with idazoxan (non-adrenoceptor, imidazoline receptor antagonist) but not by pretreatment with rauwolscine (α₂-adrenoceptor antagonist). The renal effects of 2,6-DMC were also blocked by idazoxan but not rauwolscine (Smyth & Li, 1991), indicating that 2,6-DMC and moxonidine may be acting at the same site and/or receptor.

Rilmenidine has at least a 3 fold greater affinity for I₁ non-adrenoceptor, imidazoline receptors than for α₂-adrenoceptors (Gomez et al., 1991; Ernsberger et al., 1992) and the cardiovascular action of central administration of rilmenidine was blocked by idazoxan (Felldman et al., 1990). The present study demonstrated that, like moxonidine (Allan et al., 1993) and 2,6-DMC (Smyth & Li, 1992a), rilmenidine increased urine flow rate and sodium excretion in a dose-related fashion. Similarly, the increase in flow rate was associated...
with an increase in osmolar clearance rather than an increase in free water clearance as has been documented for $\alpha_2$-adrenoceptor agonists (Blandford & Smyth, 1990; Gellai, 1990). This similarity of 2,6-DMC with moxonidine and rilmenidine would be consistent with 2,6-DMC acting at the same site, i.e. the non-adrenoceptor, imidazoline receptor. However, radioligand binding studies will be required to support this contention.

In the present study, the renal effects of rilmenidine and moxonidine were significantly attenuated in 1K-IC hypertensive rats as compared with 1K-sham normotensive rats. These results were similar to those found for 2,6-DMC in 1K-IC hypertensive rats (Li & Smyth, 1994). It is important to note that this decrease in response to non-adrenoceptor, imidazoline agonists may be specific since the natriuretic response to an $\alpha_2$-adrenoceptor agonist (Li & Smyth, 1994) and a $\alpha_2$ vasopressin receptor antagonist (Li & Smyth, 1993) were not found to be altered in 1K-IC hypertension.

During the chronic phase of 1K-1C hypertension, blood pressure is maintained by an excess of water and sodium retention (Zandberg, 1984; Nabel et al., 1985). Conceivably, the attenuated effects of renal imidazoline receptor stimulation in 1K-1C hypertensive rats could contribute to the sodium and water retention and may be caused by one of two factors. First, an increase in the endogenous levels of the non-adrenoceptor, imidazoline receptor agonist could result in a decrease in the number of these receptors available (receptor occupation) which would decrease the response to the exogenous non-adrenoceptor, imidazoline receptor agonists. At present, the endogenous agonist for non-adrenoceptor, imidazoline receptors has not been determined. However, recent studies suggest that clonidine-displacing substance may be the endogenous non-adrenoceptor, imidazoline receptor agonist (Atlas, 1991; Regunathan et al., 1991). Second, this decreased response may represent a decrease in receptor activity. Insel & Motulsky (1988) found that $\alpha_2$-adrenoceptors in tissues such as those in rat kidney may be down- or up-regulated. Antagonists induced an up-regulation and agonists mediated a down-regulation of $\alpha_2$-adrenoceptors in simultaneously hypertensive rats, DOCA-salt hypertensive rats and New Zealand Genetically Hypertensive rats (Saiz et al., 1987; Sanchez et al., 1989; Smyth et al., 1992b). The 1,

Figure 4 The effects of moxonidine on mean arterial blood pressure, heart rate and creatinine clearance. The solid columns represent the 1K-sham rats and the hatched columns represent the 1K-IC hypertensive rats. *denotes $P<0.05$ between 1K-sham normotensive and 1K-IC hypertensive rats. #denotes $P<0.05$ as compared with the respective control group.

Figure 5 The effects of moxonidine on urine flow rate and sodium excretion. The solid columns represent the 1K-sham rats and the hatched columns represent the 1K-IC hypertensive rats. *denotes $P<0.05$ between 1K-sham normotensive and 1K-IC hypertensive rats. #denotes $P<0.05$ as compared with the respective control group.
non-adrenoceptor, imidazoline receptor may also undergo similar regulation. An infusion of angiotensin II produced an up-regulation of I$_1$ sites (Ernsberger et al., 1991). Similarly, although the receptor subtype was not described, Olmos et al. (1992) demonstrated that chronic treatment with idazoxan or cirazoline (imidazoline compounds) increased the density of non-adrenoceptor, imidazoline receptors in the brain of Wistar Kyoto and Sprague-Dawley rats but not spontaneously hypertensive rats. The altered non-adrenoceptor, imidazoline receptor number/activity may be secondary to increased agonist levels (clonidine-displacing substance) or a pathophysiologically decrease in receptor activity. Recent studies in our laboratory have shown that [3H]-idazoxan binding was decreased in kidneys of 1K-1C hypertensive rats compared with 1K-sham normotensive rats. Conversely [3H]-rauwolscine binding was similar in 1K-1C hypertensive and 1K-sham normotensive rats (Li & Smyth, 1994). This decrease in sites labelled by [3H]-idazoxan in 1K-1C kidneys would be consistent with the decrease in response to non-adrenoceptor, imidazoline agonists reported in the present study.

In summary, the renal effects of rilmenidine were completely attenuated and the renal response of moxonidine was significantly decreased in 1K-1C hypertensive rats compared with the response in 1K-sham normotensive rats. This attenuation indicated that the activity of renal I$_1$ non-adrenoceptor, imidazoline receptors was decreased. Whether the decrease in activity of this natriuretic I$_1$ non-adrenoceptor, imidazoline receptor contributes to the increase in blood pressure in this 1K-1C acquired model of hypertension remains to be determined.

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References


