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RESEARCH PAPER

Influence of combined hypertension and renal failure on functional α_1-adrenoceptor subtypes in the rat kidney

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Background and purpose: This study investigated whether the α_1-adrenoceptor responsiveness of the renal vasculature was altered in the state of hypertension combined with renal failure.
Experimental approach: Male spontaneously hypertensive rats (SHR) received cisplatin (5 mg kg^{-1} i.p.) to induce renal failure. Seven days later, the rats were anaesthetized and the reductions in renal blood flow (RBF) to electrical renal nerve stimulation (RNS) and intrarenal administration of three adrenoceptor agonists (noradrenaline, phenylephrine and methoxamine) were determined before and after amlodipine, 5-methylurapidil, chloroethylclonidine or BMY 7378.
Key results: In renal failure SHR (RFSHR), RBF and creatinine clearance were significantly reduced (approximately 70%), while urine output and fractional sodium excretion were four and twenty-fold higher, respectively, compared to SHR. Vasoconstrictions induced by RNS or the adrenoceptor agonists were greater in RFSHR than SHR, and these responses were blunted by 5-methylurapidil, BMY 7378 and amlodipine in the SHR, while chloroethylclonidine had no effect. In the RFSHR, all renal vasoconstrictions were reduced by amlodipine and BMY 7378 but 5-methylurapidil attenuated those caused by RNS, noradrenaline and methoxamine while those to phenylephrine were enhanced. Chloroethylclonidine potentiated renal vasoconstrictor responses to methoxamine and phenylephrine but not RNS or noradrenaline in RFSHR.
Conclusions and implications: These findings suggest α_{1A}- and α_{1D}-adrenoceptors mediated the renal vasoconstrictor responses in SHR and RFSHR. In the RFSHR, other α_1-adrenoceptor subtypes, for example, α_{1B}-adrenoceptors appeared to play a greater role.
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Keywords: α_1-adrenoceptor; rat renal resistance vessels; renal failure; spontaneously hypertensive rats

Abbreviations: BMY 7378, 8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride; RBF, renal blood flow; RFSHR, renal failure spontaneously hypertensive rat; RNS, renal nerve stimulation; SHR, spontaneously hypertensive rat

Introduction

The sympathetic nervous system importantly regulates renal blood flow (RBF) and glomerular filtration rate and can thereby determine extracellular volume and arterial blood pressure (DiBona and Kopp, 1997; Salomonsson *et al*., 2000). Along the renal vasculature, catecholamines released from the nerve terminals activate G-protein-coupled cell surface adrenoceptors and cause contraction by virtue of their ability to increase cytosolic calcium concentration (Walsh, 1994; Salomonsson *et al*., 2000). Three subtypes of α_1-adrenoceptor subtypes, namely α_{1A}, α_{1B} and α_{1D}, have been identified, based on pharmacological and cloning studies (Guimarães and Moura, 2001; García-Sáinz and Villalobos-Molina, 2004; Muramatsu *et al*., 2006). Several *in vivo* and *in vitro* studies have revealed that, functionally, α_1-adrenoceptors predominate in the renal vasculature (Schmitz *et al*., 1981; Wolf *et al*., 1987) and in the rat, α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors have been shown to mediate catecholamine-induced constriction (Villalobos-Molina *et al*., 1997; Salomonsson *et al*., 2000) with the α_{1A}-subtype playing a dominant role (Sattar and Johns, 1994a, b). It has also been reported that both the α_{1A}- and α_{1D}-subtypes are coexpressed and mediate the functional end point (Villalobos-Molina *et al*., 1997; Salomonsson *et al*., 2000; Arévalo-León *et al*., 2003).

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It has been further suggested that in pathophysiological conditions, there is a shift in the functional contribution of α_1-adrenoceptor subtypes in certain vascular beds. In support of this notion, it was observed that with the development of diabetes there could be enhancement (Jackson and Carrier, 1981) or inhibition (Beenen *et al*., 1996) of adrenergically induced vasoconstrictions, which were largely mediated by α_1-adrenoceptors. Indeed, α_1-adrenoceptors are involved in rapid processes, such as sequestration, and slower processes such as receptor downregulation, which has been reported to be prolonged in pathophysiological states characterized by exaggerated sympathetic activity, for example, cardiac failure and chronic renal failure (Packer, 1992).

Renal sympathetic nerve activity is elevated in the spontaneously hypertensive rat (SHR) (DiBona and Kopp, 1997) but the functional α_1-adrenoceptor subtypes, which are involved in regulating RBF are similar to those found in the normotensive Wistar rat. There is increasing evidence that in the situation of renal injury, or as chronic renal failure progresses, afferent signals from the kidney appear to induce a reflex activation of the sympathetic nervous system (Campese *et al*., 2004). The exact nature of the signal activating the sensory nerves of the kidney, whether changes in tissue composition or circulating uraemic toxins, is unclear. However, the possibility arises that a state of hypertension combined with renal failure would generate an even greater level of sympathoexcitation, further contributing to a change in the composition of functional α_1-adrenoceptor subtypes mediating vasoconstriction in the kidney. To this end, this study addressed the hypothesis that a combined state of hypertension and renal failure would lead to an alteration in the contribution of the different α_1-adrenoceptor subtypes mediating constriction of the renal vasculature.

Materials and methods

Animals

Approval was obtained for all animal protocols and procedures from the Animal Ethics Committee, Universiti Sains Malaysia (Penang, Malaysia). Male SHRs were bred and maintained in the Animal House of Universiti Sains Malaysia (Penang, Malaysia) and were used at 250–300 g. They were provided with rat chow (Gold Coin Feed Mills, Penang, Malaysia) and water *ad libitum*. All animals were divided into eight groups ($n = 5$–9). Groups 1–4 were normal SHRs, whereas groups 5–8 were SHRs with renal failure.

Induction of renal failure and metabolic data collection

Rats were caged individually in custom-made stainless steel metabolic cages and acclimatized at least for 3 days prior to the induction of renal failure. Baseline metabolic data (body weight, water intake and urine output) were recorded. Animals were deprived of food for 12–16 h and on the subsequent day injected with a single i.p. dose of 5 mg kg^{-1} cisplatin (Yatsu *et al*., 2003; Kang *et al*., 2004; Mishima *et al*., 2006; Khan *et al*., 2007). Further metabolic data were collected on every alternate day over a period of 7 days and on day 7 the animals entered into the acute haemodynamic study. Plasma samples were collected on days 0 and 7 and kept frozen (−70 °C) until analysed for creatinine and sodium using spectrophotometry and flame photometry, respectively. Upon completion of the haemodynamic study, the contralateral kidney, which was not exposed to agonists or antagonist, was collected and preserved in 10% formalin for histological examination using haematoxylin and eosin staining. The kidney index was calculated as 100 × kidney weight/body weight (Hye Khan *et al*., 2007; Saad *et al*., 2007).

Haemodynamic study

Overnight (12–14 h) fasted rats were anaesthetized with 60 mg kg^{-1} i.p. sodium pentobarbitone (Nembutal; CEVA Sante Animale, Libourne, France). After tracheostomy, to provide a free airway passage to facilitate respiration, a carotid artery was cannulated and connected to a blood pressure transducer (P23 ID Gould; Statham Instruments, Oakland, CA, USA) linked to a computerized data acquisition system for the measurement of mean arterial blood pressure. The left jugular vein was cannulated to permit infusion of maintenance doses of anaesthetic as required.

A midline abdominal incision was undertaken to expose the left kidney and the renal artery was carefully cleared and fitted with an electromagnetic flow probe (EP 100 series; Carolina Medical Instruments, King, NC, USA), which was coupled to an electromagnetic flow meter (Carolina Medical Instruments) for continuous measurement of RBF. The renal haemodynamic data were recorded using a computerized data acquisition system (PowerLab; AD Instrument, Sydney, NSW, Australia).

The iliac artery was cannulated such that the tip of the cannula lay close to the entrance of the renal artery to enable exogenous administration of adrenoceptor agonists and antagonists (Sattar and Johns, 1994a, b; Armenia *et al*., 2004; Khan *et al*., 2007). The cannula was kept patent by a continuous infusion of saline at 6 ml h^{-1}. The renal nerves passing from the coeliac and aorticorenal ganglia to the kidney were isolated and carefully dissected for a short length and placed on fine bipolar stainless steel wire electrodes, which was connected to an electrical stimulator (Grass S 48 Stimulator; Grass Medical Instrument, Quincy, MA, USA). The functionality of the renal nerves was tested in terms of blanching of the kidney in response to a stimulation of the nerves at 15 V, 0.2 ms, 10 Hz for 30–60 s. At the end of the experiment the animals were killed using an overdose of anaesthetic.

Preparation of the drugs

Amlodipine (Pfizer, Groton, NY, USA), 5-methylurapidil (RBI, Natick, MA, USA), chloroethylclonidine (RBI) and BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride; RBI) were prepared either in saline (0.9% NaCl) or in lactic acid as recommended by the manufacturer and kept frozen as aliquots of stocks and diluted on the day just prior to use. The adrenoceptor agonists, noradrenaline, phenylephrine and methoxamine, were prepared in saline daily prior to use.

General experimental protocol
The entire experiment was carried out in three parts. In the initial part, saline was infused intrarenally during which the renal nerves were stimulated electrically for periods of 2 min at increasing frequencies of 1, 2, 4, 6, 8 and 10 Hz and then in the reverse order of the same frequencies; subsequently, graded bolus doses of noradrenaline (25, 50, 100 and 200 ng), phenylephrine (0.25, 0.5, 1 and 2 μg) and methoxamine (1, 2, 3 and 4 μg) were administered in ascending doses followed by descending doses. In the second part, the lower dose (bolus plus continuous infusion) of antagonist was given, and 20 min later, the sequence of renal nerve stimulation (RNS) and bolus injections of adrenoceptor agonists (noradrenaline, phenylephrine and methoxamine) were repeated. In the last part, twice the original bolus dose and continuous infusion of antagonist was administered via the close renal arterial cannula and 20 min later the nerves were stimulated and the agonist injections were repeated as described in the first two phases.

Groups 1 and 5. After the first part, the animals received a close intrarenal bolus dose of 200 $\mu g\,kg^{-1}$ plus an infusion of 50 $\mu g\,kg^{-1}\,h^{-1}$ amlodipine and in the last part twice this dose of amlodipine was used.

Groups 2 and 6. Following the first part with continuous infusion of saline, a close intrarenal bolus dose of 5 $\mu g\,kg^{-1}$ plus an infusion of 1.25 $\mu g\,kg^{-1}\,h^{-1}$ 5-methylurapidil was given after which the second set of vasoconstriction experiments were carried out. In the last part, a bolus dose of 10 $\mu g\,kg^{-1}$ plus an infusion of 2.5 $\mu g\,kg^{-1}\,h^{-1}$ 5-methylurapidil was given after which a final set of vasoconstriction experiments were undertaken.

Groups 3 and 7. Following the first part, an intrarenal a bolus dose (5 $\mu g\,kg^{-1}$) plus a continuous infusion (1.25 $\mu g\,kg^{-1}\,h^{-1}$) of chloroethylclonidine was commenced and the second set of vasoconstriction experiments were carried out. In the last phase, twice the dose used in second phase was given and the third set of vasoconstriction experiments were performed.

Groups 4 and 8. After the first set of vasoconstriction experiments, a close intrarenal bolus dose of 100 $\mu g\,kg^{-1}$ plus an infusion of 25 $\mu g\,kg^{-1}\,h^{-1}$ of BMY 7378 was administered and the second set of vasoconstriction experiments were carried out. In the last phase, twice the dose of BMY 7378 (200 $\mu g\,kg^{-1}$ and 50 $\mu g\,kg^{-1}\,h^{-1}$) was used after which the third set of vasoconstriction experiments were performed.

Statistical analysis
The responses in RBF to RNS and all the adrenoceptor agonists were taken as the difference between the baseline value immediately prior to RNS or agonist administration. The peak reductions, for both the increasing and decreasing doses or stimulation frequencies, and an average value was taken across the whole of the dose or stimulus frequency–response range as previously described (Sattar and Johns, 1994a, b; Armenia *et al*., 2004; Khan *et al*., 2007). Data were expressed as mean ± s.e.mean. Statistical analyses of the haemodynamic, metabolic and other data were performed using either two-way or one-way ANOVA followed by the Bonferroni *post hoc* test. The difference between the means was considered significant at the 5% level. All statistical analyses were performed using the Superanova statistical package (Abacus, Berkeley, CA, USA).

Results

General observations
In the renal failure spontaneously hypertensive rat (RFSHR), the degree of renal functional deterioration was characterized in terms of an approximate 70% reduction in creatinine clearance, a significant 20-fold increase in fractional sodium excretion, a significant increase of some 20% in the kidney index and a 4- to 5-fold increase in 24 h urine output (Tables 1 and 2). There was notable renal tubular damage, primarily affecting the epithelial cells, in the RFSHR as compared with the normal SHR as can be seen in Figure 1. Baseline mean arterial blood pressure was similar in both experimental groups but was higher by about 10% in the SHR as compared with that of the RFSHR (Table 2). Basal RBF during the acute studies was significantly ($P<0.05$) lower, by approximately 70%, in RFSHR compared with SHR (Table 2). In the acute studies, it was observed that RBF in the SHR was 17.6 ± 1.4 $ml\,min^{-1}\,kg^{-1}$, whereas it was markedly lower in the RFSHR at 8.6 ± 0.7 $ml\,min^{-1}\,kg^{-1}$. Administration of amlodipine or the adrenoceptor antagonists had no effect on RBF in any of the SHR or RFSHR groups at either dose.

Vasoconstrictor responses
Figure 2 shows that the renal vasoconstrictor responses to graded RNS and to the increasing doses of noradrenaline, phenylephrine and methoxamine in the SHR and RFSHR in the saline-treated control phase, expressed as percentage changes. It can be seen that over the stimulus frequency/

Table 1 Renal functional parameters in spontaneously hypertensive rats (SHRs) and renal failure spontaneously hypertensive rats (RFSHRs)

Animal group	*Kidney index*	*PNa (mM)*	*PCr ($\mu g\,ml^{-1}$)*	*FE_{Na} (%)*	*CCr ($ml\,min^{-1}\,100\,g^{-1}$)*
SHRs ($n=18$)	0.41 ± 0.02	141.6 ± 1.9	8.3 ± 0.7	0.50 ± 0.04	0.9 ± 0.22
RFSHRs ($n=18$)	0.66 ± 0.01*	109.7 ± 3.4*	28.8 ± 3.6*	10.00 ± 0.78*	0.3 ± 0.01*

Kidney index = (kidney weight body weight^{-1}) × 100. Values for plasma sodium (PNa), plasma creatinine (PCr), urinary sodium (UNa), urinary creatinine (UCr), fractional excretion of sodium (FE_{Na}) and creatinine clearance (CCr) are shown.
All data were analysed by two-way ANOVA followed by Bonferroni *post hoc* test and presented as mean ± s.e.mean.
*Significant difference between SHR and RFSHR; $P<0.05$.

Table 2 General observations in spontaneously hypertensive rats (SHRs) and renal failure spontaneously hypertensive rats (RFSHRs)

Experimental groups	*BWt (g)*	*UOP (ml 24 h^{-1})*	*WI (ml 24 h^{-1})*	*Baseline MAP (mm Hg)*	*Baseline RBF (ml min^{-1} kg^{-1})*
SHRs ($n=24$)	284.6 ± 8	7.1 ± 0.7	31.6 ± 3.4	142 ± 2	18 ± 1
RFSHRs ($n=24$)	269 ± 6*	32.2 ± 1.1*	23 ± 1.2*	130 ± 3	6 ± 1*

Abbreviations: BWt, body weight; UOP, 24 h urine output; WI, 24 h water intake; MAP, baseline mean arterial pressure; RBF, baseline renal blood flow.
Data presented here as mean ± s.e.mean.
*Significant difference between SHR and RFSHR; $P<0.05$.

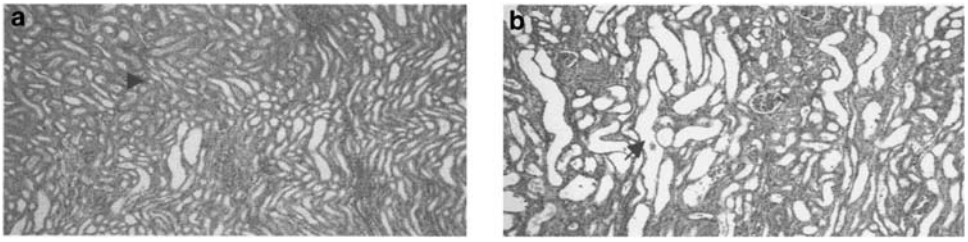

Figure 1 Representative photomicrographs of light microscopy of renal tissue (5 μm) from spontaneously hypertensive rats (SHRs; **a**), cisplatin-induced renal failure spontaneously hypertensive rats (RFSHRs; **b**). Haematoxylin and eosin staining (× 200). The arrows indicate normal and damaged tubules in the normal and renal failure animals, respectively. In the RFSHRs, there was a severe disruption of the tubular structures.

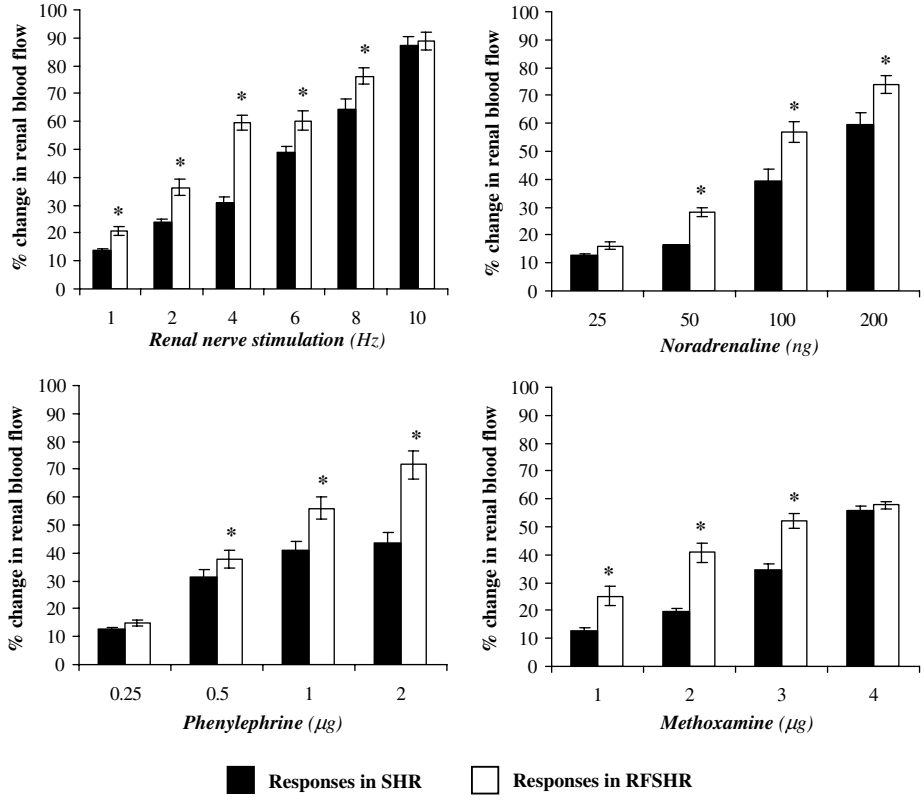

Figure 2 Mean percentage decrease in renal blood flow (RBF) in response to adrenergic stimuli in normal spontaneously hypertensive rats (SHRs) and renal failure spontaneously hypertensive rats (RFSHRs). *$P<0.05$, SHR vs RFSHR. The data are presented as mean percentage decrease ± s.e.mean. ($n=8$). Data were analysed by two-way ANOVA followed by Bonferroni *post hoc* test.

dose–response curves, the renal vasoconstrictions were larger in the RFSHR than the SHR groups.

Renal nerve stimulation

The data in Figure 3 present the mean percentage change averaged for the whole of the stimulus frequency–response curve during either saline infusion, the low or high dose of antagonist. It can be seen that in the RFSHR and SHR groups, the L-type calcium channel blocker amlodipine attenuated the neurally induced renal vasoconstriction in a dose-dependent way. The α_{1A}-adrenoceptor antagonist 5-methylurapidil also blunted the magnitude of the RNS-induced renal vasoconstrictor responses in both the RFSHR and SHR to a similar degree in both experimental groups (Figure 3). Intrarenal administration of chloroethylclonidine

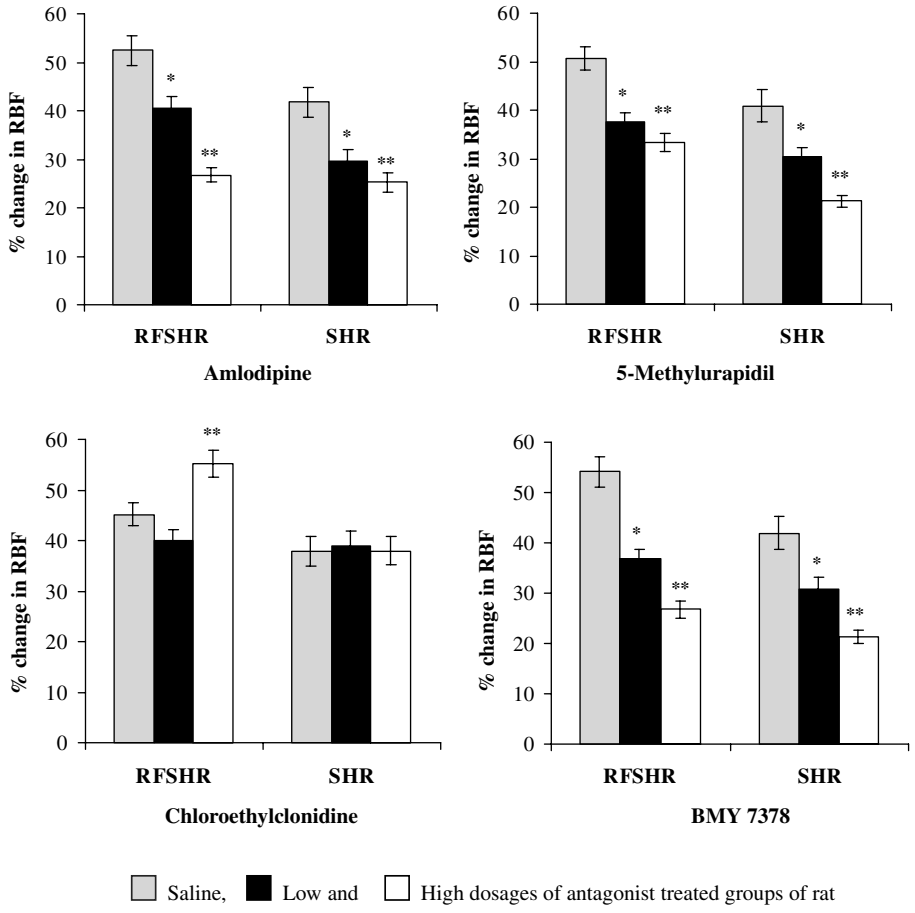

Figure 3 Renal nerve stimulation-induced renal vasoconstrictor responses in normal spontaneously hypertensive rats (SHRs) and renal failure spontaneously hypertensive rats (RFSHRs) in the presence and absence of amlodipine, 5-methylurapidil, chloroethylclonidine and BMY 7378. Data presented as mean ± s.e.mean ($n = 6$) and were analysed by two-way ANOVA followed by Bonferroni *post hoc* test. *Indicates significant ($P < 0.05$) difference between saline- and low-dose-treated groups. **Indicates significant ($P < 0.05$) difference between saline- and high-dose-treated groups. BMY 7378, 8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride.

significantly enhanced the RNS-induced renal vasoconstrictor responses in RFSHR at high doses. By contrast, in the SHR rats, chloroethylclonidine did not cause any meaningful alteration in the magnitude of the RNS-induced renal vasoconstrictor responses (Figure 3). In the RFSHR and SHR, the RNS-induced renal vasoconstrictor responses were significantly blunted by BMY 7378, to a similar degree and with a comparable pattern (Figure 3).

Noradrenaline

The noradrenaline-induced renal vasoconstrictor responses in both the RFSHR and SHR were significantly blunted by amlodipine in a dose-related manner (Figure 4). A similar dose-dependent significant attenuation of the noradrenaline-induced renal vasoconstrictor responses was exhibited when 5-methylurapidil was given to the RFSHR and SHR groups (Figure 4). In both hypertensive and renal failure rats, chloroethylclonidine at both doses had no effect on the magnitude of the noradrenaline-induced renal vasoconstrictor responses (Figure 4). The noradrenaline-induced renal vasoconstrictor responses in the RFSHR and SHR groups were significantly blunted by BMY 7378, the magnitude of which was dose related (Figure 4).

Phenylephrine

Intrarenal administration of phenylephrine caused dose-related renal vasoconstrictor responses in both the RFSHR and SHR groups, which were significantly blunted by amlodipine with a comparable pattern and to a similar degree (Figure 5). 5-Methylurapidil also caused a significant dose-dependent attenuation of the magnitude of the phenylephrine-induced renal vasoconstrictor responses in the SHR (Figure 5). By contrast, in the RFSHR, the low-dose 5-methylurapidil had no effect on the renal vasoconstrictor responses to phenylephrine, but following the higher dose of 5-methylurapidil, there was a significant enhancement of the renal vasoconstrictor responses (Figure 5). In the SHR, neither dose of chloroethylclonidine had any effect on the phenylephrine-induced renal vasoconstrictor responses. By contrast, in the RFSHR, the phenylephrine-induced renal vasoconstrictor responses were unchanged during the administration of the low dose of chloroethylclonidine but were significantly augmented during administration of the

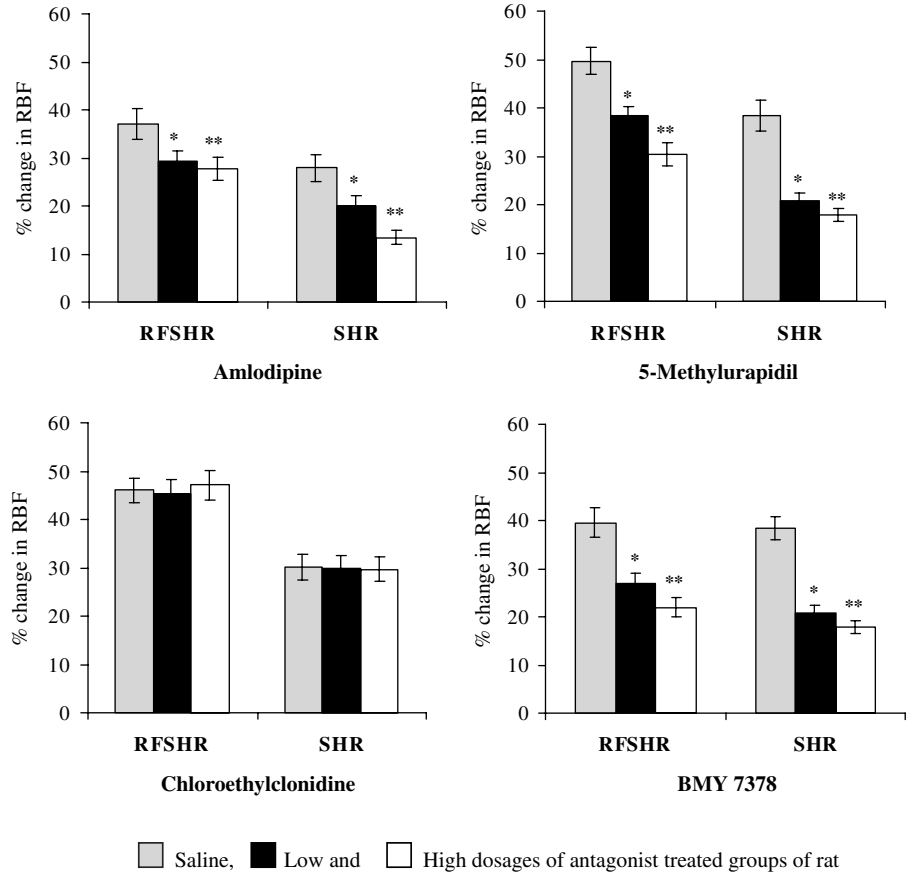

Figure 4 Noradrenaline-induced renal vasoconstrictor responses in normal spontaneously hypertensive rats (SHRs) and renal failure spontaneously hypertensive rats (RFSHRs) in the presence and absence of amlodipine, 5-methylurapidil, chloroethylclonidine and BMY 7378. Data presented as mean ± s.e.mean ($n = 6$) and were analysed by two-way ANOVA followed by Bonferroni *post hoc* test. *Indicates significant ($P < 0.05$) difference between saline- and low-dose-treated groups. **Indicates significant ($P < 0.05$) difference between saline- and high-dose-treated groups. BMY 7378, 8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride.

high dose (Figure 5). The α_{1D}-adrenoceptor-selective antagonist BMY 7378 significantly depressed the magnitude of the phenylephrine-induced renal vasoconstrictor responses in both RFSHR and SHR in a dose-related manner and to a comparable extent (Figure 5).

Methoxamine

The magnitude of the methoxamine-induced renal vasoconstrictor responses were significantly attenuated by amlodipine in both the RFSHR and the SHR groups of rats (Figure 6). It was observed that in the RFSHR and SHR groups that the methoxamine-induced renal vasoconstrictor responses (Figure 6) were significantly blunted by 5-methylurapidil in a dose-related way and to a similar degree. Chloroethylclonidine at the high dose, significantly enhanced methoxamine-induced renal vasoconstrictor responses in the RFSHR, while the magnitude of these responses were unchanged during the low dose of chloroethylclonidine (Figure 5). By contrast, in the SHR the magnitude of the renal vasoconstrictions induced by methoxamine were unchanged in the presence of both the low and high doses of chloroethylclonidine (Figure 6). The α_{1D}-adrenoceptor-selective antagonist BMY 7378 (Figure 6) significantly attenuated the magnitude of the methoxamine-induced renal vasoconstrictor responses in both the RFSHR and SHR groups.

Discussion

The renal circulation is richly endowed with sympathetic nerves, which allow the autonomic nervous system to play an important role in the regulation of renal haemodynamic and tubular function and thereby body fluid homeostasis and blood pressure (Barajas *et al*., 1992; DiBona and Kopp, 1997). Pharmacological, molecular biological and functional studies have shown that three subtypes of α_1-adrenoceptors, α_{1A}-, α_{1B}- and α_{1D}-, are present in the kidney (Salomonsson *et al*., 2000; Guimarães and Moura, 2001; Muramatsu *et al*., 2006) with α_{1A}-adrenoceptors being the predominant subtype in the vasculature. It has been reported that, in different pathophysiological states, the functional contribution of the different α_1-adrenoceptor subtypes (Stassen *et al*., 1998; Armenia *et al*., 2004; Khan *et al*., 2007) can change. The aim of this investigation was to determine whether a comparable

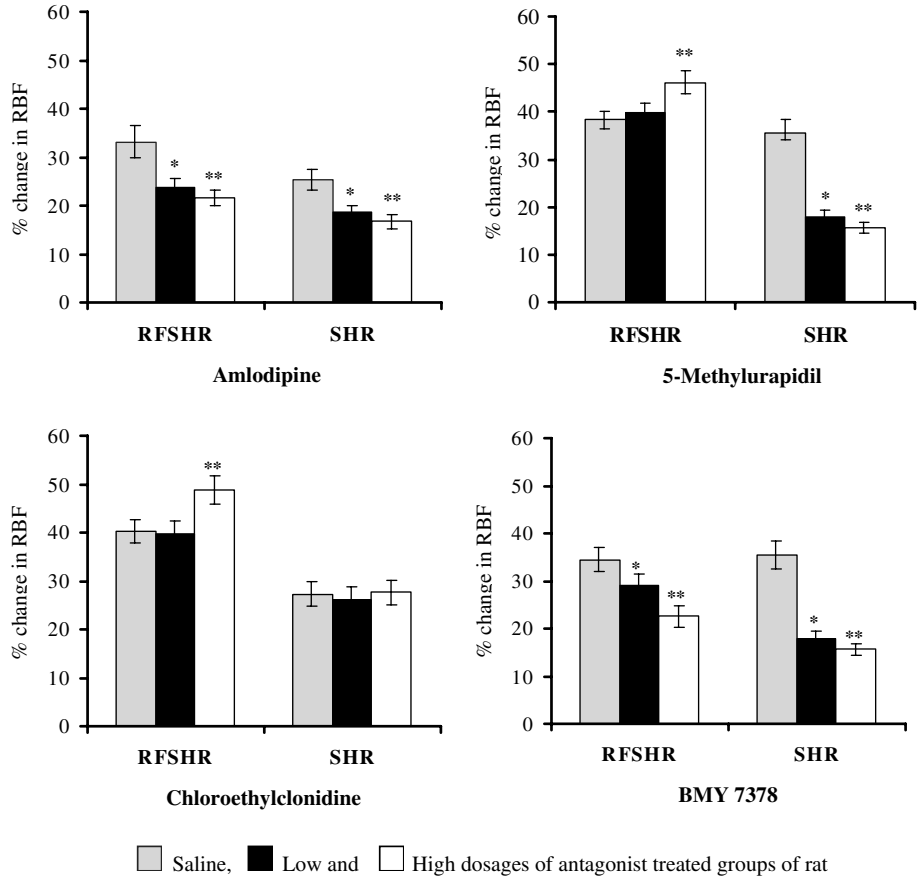

Figure 5 Phenylephrine-induced renal vasoconstrictor responses in normal spontaneously hypertensive rats (SHRs) and renal failure spontaneously hypertensive rats (RFSHRs) in the presence and absence of amlodipine, 5-methylurapidil, chloroethylclonidine and BMY 7378. Data presented as mean ± s.e.mean ($n = 6$) and were analysed by two-way ANOVA followed by Bonferroni *post hoc* test. *Indicates significant ($P < 0.05$) difference between saline- and low-dose-treated groups. **Indicates significant ($P < 0.05$) difference between saline- and high-dose-treated groups. BMY 7378, 8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride.

change in α_1-adrenoceptor subtype functionality occurred when renal failure was induced in genetically hypertensive rats.

Cisplatin (5 mg kg^{-1}) administration resulted in reductions in body weight and water intake, a marked increase in urine output together with a fall in creatinine clearance indicative of a decreased glomerular filtration, which were all comparable to that reported by others (Yatsu *et al*., 2003; Kang *et al*., 2004; Shimeda *et al*., 2005). Moreover, there was more than 20-fold rise in fractional sodium excretion, which was indicative of a marked reduction in reabsorptive capacity and was probably a consequence of damage to the tubular epithelial cells of the nephron. Indeed, this latter view is supported by the histological observations, where the tubules showed clear destruction of their structural integrity. Blood pressure was also lower in the RFSHR, which has been reported by others (Matsushima *et al*., 1998; Bagnis *et al*., 2001), and one possibility is that the excessive loss of fluid via the urine (Table 1) would have depleted the extracellular fluid volume and hence caused a decrease in blood pressure.

Basal RBF in the anaesthetized RFSHR was approximately one-third that of the normal SHR yet in proportionate terms, the renal vasoconstrictor responses were almost always greater compared with normal SHR rats (Figure 2). This relatively greater responsiveness to RNS and adrenoceptor agonists in the renal failure rats could be due to an activation of the sympathetic nervous system that has been reported to occur in rats and humans with renal failure (Boero *et al*., 2001; Rump, 2001; Campese and Krol, 2002; Ciriello and De Oliveira, 2002; Phillips, 2005). These observations were in accord with findings of earlier studies in 2K1C Goldblatt rats (Sattar and Johns, 1994a), genetically hypertensive rats, diabetic rats, rats with a combined state of diabetes and spontaneous hypertension (Armenia *et al*., 2004), and in rats with heart failure (Abbas *et al*., 2006, 2007).

It was observed that neither amlodipine nor any of the selective α_1-adrenoceptor antagonists had any effect on basal renal haemodynamics in either the SHR or RFSHR. Moreover, these agents had no effect on the systemic circulation as there was no change in the blood pressure before and after they were administered intrarenally. Amlodipine, BMY 7378 and 5-methylurapidil attenuated, but did not completely block the renal vasoconstrictor responses in the SHR rats elicited by RNS and exogenous administration of adrenoceptor agonists (noradrenaline, phenylephrine and methoxamine), which suggested a contribution from multiple

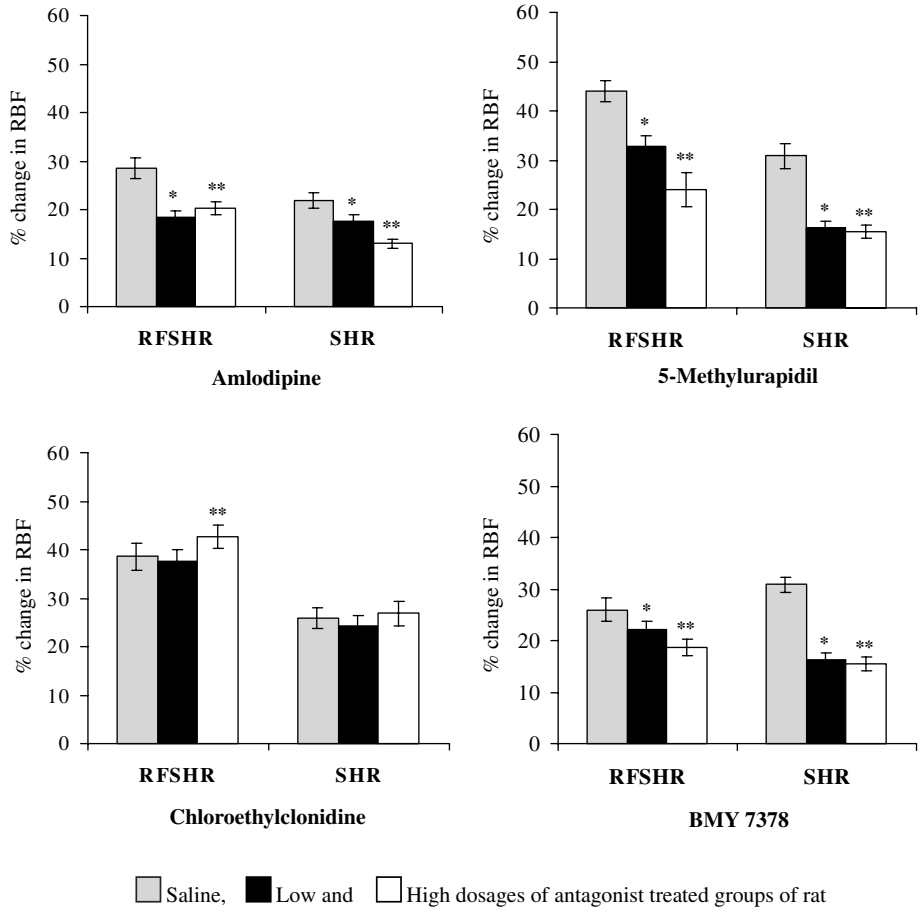

Figure 6 Methoxamine-induced renal vasoconstrictor responses in normal spontaneously hypertensive rats (SHRs) and renal failure spontaneously hypertensive rats (RFSHRs) in the presence and absence of amlodipine, 5-methylurapidil, chloroethylclonidine and BMY 7378. Data presented as mean ± s.e.mean ($n = 6$) and were analysed by two-way ANOVA followed by Bonferroni *post hoc* test. *Indicates significant ($P < 0.05$) difference between saline- and low-dose-treated groups. **Indicates significant ($P < 0.05$) difference between saline- and high-dose-treated groups. BMY 7378, 8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride.

α_1-adrenoceptor subtypes. The observations were consistent with the involvement of α_{1A}- and α_{1D}-adrenoceptor subtypes, which were compatible with several earlier studies reporting the presence of multiple α_1-adrenoceptor subtypes in the renal vasculature (Villalobos-Molina and Ibarra, 1996; Villalobos-Molina *et al*., 1997; Salomonsson *et al*., 2000). Chloroethylclonidine caused no alteration in the RNS- or adrenoceptor agonist-induced renal vasoconstrictor responses in the SHR, which would be consistent with there being a relatively low contribution of the α_{1B}-adrenoceptor subtypes in the renal vasculature of these rats.

In the case of RFSHR, amlodipine, 5-methylurapidil and BMY 7378 attenuated the RNS- and adrenergically induced renal vasoconstrictor responses, as effectively as in the SHR, which would indicate α_{1A}- and α_{1D}-adrenoceptor subtypes being the functional receptors. The contribution of multiple α_1-adrenoceptor subtypes was further supported by the fact that 5-methylurapidil enhanced the phenylephrine-induced responses as 5-methylurapidil is selective antagonist of α_{1A}-adrenoceptor subtype and phenylephrine is effective at all the three α_1-adrenoceptor subtypes. This situation, to a degree, was very similar to that reported earlier in normotensive rats with cisplatin-induced renal failure (Khan *et al*., 2007), where multiple α_1-adrenoceptor subtypes were involved in causing renal vasoconstriction.

The observation in the RFSHR, but not the SHR, that chloroethylclonidine enhanced the renal vasoconstrictor responses evoked by RNS could be a result of chloroethylclonidine-alkylating presynaptic α_{1B}-adrenoceptors (Docherty and O'Rourke, 1997; Ibarra *et al*., 2000) that would remove their autoinhibitory action at the neuroeffector junction in response to RNS. The situation in the normotensive rats was comparable in that following the development of renal failure there was evidence for an increased contribution of prejunctional α_1-adrenoceptors (Khan *et al*., 2007) in response to RNS. It is unclear at present as to which of these potential mechanisms may be called into play.

A further consequence of the blockade of α_{1B}-adrenoceptor subtypes in the RFSHR was a raised responsiveness of the remaining α_1-adrenoceptor subtypes, and this was reflected in the enhancement by chloroethylclonidine of the renal vasoconstrictor responses induced by RNS, phenylephrine

and methoxamine. An alternative explanation of the increased magnitude of the responses to RNS, phenylephrine and methoxamine could be due to a greater participation of α_{1A}-adrenoceptor subtype, which is relatively resistant to chloroethylclonidine, whereas the α_{1D}-adrenoceptor subtype readily phosphorylates in states of sympathetic overactivity (García-Sáinz *et al*., 2001; Arévalo-León *et al*., 2003). This view would be supported by the potentiation of renal vasoconstrictor responses in other pathophysiological states, for example, the SHRs, diabetic hypertensive rats and 2K1C Goldblatt hypertensive rats (Sattar and Johns, 1994b; Armenia *et al*., 2004).

The potential contribution of spare receptors may also help to explain the enhanced responses in the presence of chloroethylclonidine as this may occur following blockade of any particular subtype of α_1-adrenoceptors, and here, it could be the α_{1B}-adrenoceptor subtype (Piascik *et al*., 1991; Arévalo-León *et al*., 2003). Furthermore, it is possible that the chloroethylclonidine mediated enhancement of the RNS-, phenylephrine- and methoxamine-induced responses was due to the characteristics of chloroethylclonidine of being able to act as a partial agonist at α_{1A}- and α_{1D}-adrenoceptor subtypes in some vasculatures. However, in the present study, this is unlikely to be the situation, as α_{1A}-adrenoceptor subtypes are resistant to the action of chloroethylclonidine, and in the face of a possible sympathetic overactivity in pathological states the α_{1D}-adrenoceptor subtype might be phosphorylated (García-Sáinz *et al*., 2001; Arévalo-León *et al*., 2003). Against this background, it could be that the enhanced vasoconstrictor responses in the RFSHR was due to the action of RNS, phenylephrine and methoxamine on α_{1A}-adrenoceptors that are unaffected by chloroethylclonidine. Moreover, it should be noted that this pattern of enhanced renal vasoconstrictor responses following chloroethylclonidine was only observed in the RFSHR, and this situation was also observed in the normotensive state in cisplatin-induced renal failure (Khan *et al*., 2007), and hence, could be due to the pathophysiological state of the rats.

In conclusion, the hypothesis was examined whether hypertension combined with renal failure altered the contribution of the different α_1-adrenoceptor subtypes mediating adrenergically induced renal vasoconstrictions. The findings demonstrated that both α_{1A}- and α_{1D}-adrenoceptor subtypes were the major functional subtypes mediating adrenergically induced vasoconstriction in the kidneys of SHR and RFSHR. The impact of a combined hypertension and renal failure resulted in a shift in the functional contribution of α_1-adrenoceptor subtypes in terms of a minor involvement of prejunctional α_1-adrenoceptors. A similar pattern of findings was also reported in the normotensive Wistar Kyoto renal failure rats (Khan *et al*., 2007), which suggests that it is renal failure *per se*, which is instrumental in causing the alteration in the functional subtypes of rat renal α_1-adrenoceptors.

Conflict of interest

The authors state no conflict of interest.

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