Acta Pharmacol Sin 2008 Feb; 29 (2): 193–203

Blackwell Publishing

Full-length article

# $\alpha_{1B}$-Adrenoceptors mediate adrenergically-induced renal vasoconstrictions in rats with renal impairment

Md Abdul Hye KHAN[1,4], Munavvar Abdul SATTAR[1], Nor Azizan ABDULLAH[2], Edward James JOHNS[3]

*[1]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia;[2]Department of Pharmacology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia; [3]Department of Physiology, Aras Windle University College Cork, Ireland*

## Key words

$\alpha_{1B}$-adrenoceptor; chloroethylclonidine; renal resistance vessels; rats

[4] Correspondence to Mr Md Abdul Hye KHAN.
Phn 60-4653-2162
E-mail abdul_hye_khan@yahoo.com

Received 2007-06-28
Accepted 2007-08-22

doi: 10.1111/j.1745-7254.2008.00727.x

## Abstract

**Aim:** This study examined whether $\alpha_{1B}$-adrenoceptors are involved in mediating adrenergically-induced renal vasoconstrictor responses in rats with pathophysiological and normal physiological states. **Methods:** Male Wistar Kyoto and spontaneously hypertensive rats were induced with acute renal failure or experimental early diabetic nephropathy by cisplatin or streptozotocin, respectively. Cisplatin-induced renal failure was confirmed by impaired renal function and pronounced tubular damage. Experimental early diabetic nephropathy was confirmed by hyperglycemia, changes in physiological parameters, and renal function. The hemodynamic study was conducted on anesthetized rats after 7 d of cisplatin (renal failure) and 4 weeks of streptozotocin (experimental early diabetic nephropathy). **Results:** In the rats with renal failure and experimental early diabetic nephropathy, there were marked reductions in their baseline renal blood flow ($P<0.01$). The baseline mean arterial blood pressure was either unaltered or lower (all $P>0.05$) in the renal failure and experimental early diabetic nephropathy rats, respectively, as compared to their non-renal failure and non-diabetic nephropathy controls. In the rats with renal impairment, chloroethylclonidine caused either accentuation or attenuation (all $P<0.01$) of the renal vasoconstrictor responses elicited by the adrenergic stimuli. However, in the non-renal failure and in the non-diabetic nephropathy rats, chloroethylclonidine did not cause any alteration in such responses ($P>0.05$). **Conclusion:** This study demonstrated the presence of functional $\alpha_{1B}$-adrenoceptors that mediated the adrenergically-induced renal vasoconstrictions in rats with renal impairment, but not in rats with normal renal function.

## Introduction

The sympathetic nervous system plays an important role in the control of renal hemodynamics[1,2]. In renal vasculature, the catecholamines released from the nerve terminals activate G protein-coupled cell surface adrenoceptors present on the cell surface and cause contraction[3,2]. Three subtypes of $\alpha_1$-adrenoceptor subtypes, namely $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$, have been documented based on pharmacological and cloning studies[4–7]. These subtypes are closely related to each other in terms of amino acid sequence, ligand affinity, and also with respect to irreversible blockade by certain alkylating agents[8]. Several *in vivo* and *in vitro* studies have revealed that, functionally, $\alpha_1$-adrenoceptors predominate in the renal vasculature[9–11]. In rat renal vasculature, all of the 3 subtypes of $\alpha_1$-adrenoceptors have been shown to mediate catecholamine-induced constriction[2,9–11] along with a dominant role of the $\alpha_{1A}$-subtype[10,11]. Some reports have also shown the functional involvement of other subtypes, such as $\alpha_{1D}$-adrenoceptors, in renal vasculature[2,12–14].

The $\alpha_{1B}$-adrenoceptors are also involved in mediating vasoconstrictions as observed in *in vitro* studies with the mesenteric resistance artery and tail artery of rats[15,16]. This subtype is also present in the renal resistance vessel and receptor binding. The RNase protection assay revealed the

expression of $\alpha_{1B}$-adrenoceptors in the renal microvessels of Wistar Kyoto and spontaneously hypertensive rats. However, there is no report on the functional contribution of this subtype in modulating adrenergically-induced renal vasoconstriction except reports on its minor involvement. A minor functional involvement of $\alpha_{1B}$-adrenoceptors in mediating renal vasoconstriction is reported in rats with some altered physiological or pathological states. In diabetes, hypertensive diabetes, heart failure, and renal failure, a minor involvement of this receptor subtype has been reported in mediating renal adrenergic responsiveness in rats[4,17,18]. Some pathological states are indeed found to influence the functional contribution of $\alpha_1$-adrenoceptors, including the $\alpha_{1B}$-subtype, in rat renal vasculature[14,18,19]. However, there is still an apparent paucity of information on the functional involvement of $\alpha_{1B}$-adrenoceptors in modulating renal vasoconstrictions that could strengthen this view. It is particularly true in terms of further study of this interesting observation in some other pathological states.

The present study investigated the influence of some pathological states with renal impairment on the functional presence of $\alpha_{1B}$-adrenoceptors in terms of its selective affinity for chloroethylclonidine. The results of this study will provide further evidence on the pathological state-dependent involvement of $\alpha_{1B}$-adrenoceptors in modulating adrenergically-induced renal vascular tone in rats.

## Methods and materials

**Animals** Male Wistar Kyoto and spontaneously hypertensive rats with (body weight range: 250–300 g, 267±8.3 g) were supplied with commercial rat chow and water *ad libitum*. They were maintained in the Animal Care Facility, Universiti Sains Malaysia, Penang, Malaysia. Animal handling and all animal procedures were approved by the Animal Ethics Committee, Universiti Sains Malaysia. The animals were randomly divided into 6 groups. Groups 1 and 2 ($n$=5–9 rats in each group) were normal and renal failure Wistar Kyoto rats, groups 3 and 4 ($n$=5–9 rats in each group) were normal and renal failure spontaneously hypertensive rats, and groups 5 and 6 ($n$=5–9 rats in each group) were rats with experimental early diabetic nephropathy and the non-diabetic nephropathy control group, respectively.

**Induction of renal failure and physiological data collection** The animals were caged individually in custom-built stainless steel metabolic cages and acclimatized for at least 3 d before the induction of renal failure with cisplatin. Baseline physiological data (body weight, 24 h water intake, and urine output) were recorded. The animals fasted for at least 12 h and on the following day received a single intraperitoneal injection of cisplatin (55 mg/kg)[20–22]. Further physiological data were collected on every alternate day until the animals were used in the acute renal hemodynamic study on d 7. Tail vein blood samples were collected on d 0 and 7, and plasma were separated and frozen (-70 ºC) until analyzed for creatinine and sodium using spectrophotometry and flame photometry, respectively. The kidney index was calculated as 100×kidney weight/body weight[23–25]. The kidney tissues were preserved in 10% formalin for the histological examinations using conventional hematoxylin and eosin staining followed by the analysis of micrographs (Leica image analyzing system, London, UK). Apart from the examinations of the kidney index and histological study of the kidney, renal failure and impairment of renal functions in these rats were assessed from plasma creatinine, creatinine clearance, fractional excretion of sodium, and the glomerular filtration rate.

**Preparation of experimental early diabetic nephropathy rats and physiological data collection** The rats with experimental early diabetic nephropathy were developed using spontaneously hypertensive rats by a slightly modified method described earlier by several researchers[25,26–28]. In this approach, the spontaneously hypertensive rats were treated with a single intraperitoneal injection (55 mg/kg) of streptozotocin after 12–16 h of fasting. On d 3 of post-streptozotocin injection fasting ($\geq$12 h), blood glucose was tested (between 9:00–9:30) to confirm the diabetic state. Rats with a fasting blood glucose level of >13.8 mmol/L were considered to be diabetic[25,27,29–30]. The rats with confirmed diabetes were then randomly allotted in different experimental groups according to the experimental design. Blood glucose was tested once per week over a period of 4 weeks to observe their glycemic status. Rat with blood glucose less than 300 mg/dL (<13.8 mmol/L) were excluded from the study[27,30].

Apart from elevated blood glucose, other physiological changes, such as polyuria and a reduction in the body weight, were also considered in selecting the diabetic animals. The diabetic rats were kept for 28 d for weekly physiological data collection and also for the development of changes in renal functional parameters, such as creatinine clearance, fractional excretion of sodium, glomerular filtration rate, and the urinary albumin excretion rate. Creatinine and sodium in the plasma and urine were measured using spectrophotometry and flame photometry, respectively, and urinary albumin was measured using ELISA[31]. Finally, on d 29 the rats were used in the acute study for renal hemodynamic data collection. After the hemodynamic study, the kidney tissues were collected, the weight of the kidneys was recorded

for the determination of the kidney index (kidney index=100×kidney weight/body weight), and the kidneys were then preserved in 10% formalin for the histological examinations.

**Hemodynamic study**

Surgical preparation of the animal The overnight (≥12 h)-fasted (with water *ad libitum*) rats were anaesthetized with 60 mg/kg (ip) sodium pentobarbitone (Nembutal, CEVA Sante Animale, Libourne, France). After a tracheostomy with endotracheal cannula (PP240, Protex, Kent, England) the carotid artery was cannulated (PE 50, Protex, England) and connected to a pressure transducer (P23 ID Gould, Statham Instruments, Oakland, CA, USA) coupled to a computerized data acquisition system (PowerLab, AD-Instruments, Sydney, NSW, Australia) for the continuous measurement of the mean arterial blood pressure. The left jugular vein was cannulated (PE 50, Protex, England) to permit the infusion of maintenance doses of anesthesia.

The left kidney was exposed using a midline abdominal incision. The renal artery was carefully cleared and fitted with an electromagnetic flow probe (EP 100 series, Carolina Medical Instruments, King, North Carolina, USA) that was connected to an electromagnetic flow meter (Carolina Medical Instruments, USA) for the continuous measurement of renal blood flow. A cannula (PE 50, Protex, England) was inserted via the iliac artery so that its beveled tip lay close to the entrance of the renal artery to enable the exogenous administration of adrenergic agonists and antagonists[10,11]. The cannula was kept patent by the continuous infusion of saline at a rate of 6 $mL \cdot kg^{-1} \cdot h^{-1}$. The renal nerves passing from the coeliac and aortico-renal ganglia to the kidney were isolated and carefully dissected for a short length and placed on fine bipolar stainless steel wire electrodes. The functionality of the renal nerves was tested by stimulating (Grass S 48 Stimulator, Grass Instruments, Quincy, MA, USA) them at 15 V, 0.2 ms, and 1–10 Hz for 30 s to observe whether blanching of the kidney occurred. At the end of the experiment, the animals were euthanized by an overdose of anesthetic (sodium pentobarbitone; Nembutal, CEVA Sante Animale, France), and an autopsy was done followed by the disposal of the animal carcasses in accordance with the guidelines of the Animal Ethics Committee of the Universiti Sains Malaysia.

Experimental protocol The experiment was comprised of 3 phases. In the first phase, saline was infused continuously and intrarenally during which the renal nerves were stimulated at increasing frequencies of 1, 2, 4, 6, 8, and 10 Hz and then in the reverse order. Subsequently, graded bolus doses of noradrenaline (25, 50, 100, and 200 ng; Sanofi Winthrop, Guildford, UK), phenylephrine (0.25, 0.5, 1, and 2 μg; Knoll, Nottingham, UK), and methoxamine (1, 2, 3, and 4 μg; Wellcome, London, UK) were administered in ascending and then descending doses. After the first phase, a close intrarenal administration of a bolus dose (5 μg/kg) of chloroethylclonidine (Sigma, St Louis, MO, USA) was given followed by a continuous infusion of 1.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$. Twenty minutes later, the second set of vasoconstriction experiments were carried out. In the last phase, a bolus dose of 10 μg/kg chloroethylclonidine plus a continuous infusion of 2.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ was administered. Twenty minutes latter the last set of vasoconstriction experiments were carried out as described earlier[10,11].

Renal vasoconstrictor responses The vasoconstrictor responses were recorded as the percentage changes of renal blood flow in relation to the baseline values during graded frequencies of renal nerve stimulation and graded doses of agonist administered. The responses were recorded using a computerized data acquisition system (PowerLab, ADInstruments, Australia).

Adrenergic agonist and antagonists Chloroethylclonidine (*N*-β-chloroethyl-*N*-methylamino-methylclonidine), a selective antagonist of $\alpha_{1B}$-adrenoceptors and the most widely used agent in characterizing $\alpha_{1B}$-adrenoceptors, was used in this study for characterizing the functional involvement of $\alpha_{1B}$-adrenoceptors in mediating adrenergically-induced renal vasoconstriction, and can differentiate between $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes[32]. Regarding the $\alpha_1$-adrenoceptors, the effects of chloroethylclonidine have been related to the alkylation and inactivation of the $\alpha_1$-adrenoceptor subtypes as irreversible antagonist in the order of sensitivity of the $\alpha_{1B} \geq \alpha_{1D} >> \alpha_{1A}$-adrenoceptor subtypes[14,33]. Chloroethylclonidine was prepared in saline as recommended by the manufacturer and kept as aliquots of frozen stock and diluted prior to use.

The adrenergic agonists used were noradrenaline, phenylephrine, and methoxamine. Noradrenaline is a mixed agonist that acts on both the $\alpha_1$ and $\alpha_2$-adrenoceptors; phenylephrine is a non-selective agonist of $\alpha_1$-adrenoceptors with an ability to activate $\alpha_{1A}$-, $\alpha_{1B}$-, and $\alpha_{1D}$-adrenoceptor subtypes[14]; and methoxamine is a relatively selective $\alpha_{1A}$-adrenoceptor-subtype agonist[13,14], but may activate $\alpha_{1D}$-adrenoceptors as it does not exhibit selectivity between $\alpha_{1A}$- and $\alpha_{1D}$-adrenoceptors[13]. Noradrenaline, phenylephrine, and methoxamine were prepared fresh in saline (150 mmol/L NaCl) from frozen stocks daily prior to use.

**Statistical analysis** The renal blood flow responses caused by renal nerve stimulation, noradrenaline, phenylephrine, and methoxamine were taken as the average values caused by each dose/frequency of adrenergic stimuli administered and applied in ascending and descending orders. The overall

mean response for each dose or frequency was taken as the average value of vasoconstrictor responses (drop in renal blood flow) obtained at each level of frequency for renal nerve stimulation and each dose of the adrenergic agonists used. The data on the drop of renal blood flow were expressed as the percentage drop on the renal blood flow in relation to the basal values of renal blood flow calculated at the beginning of the administration of each stimulus (renal nerve stimulation and adrenergic agonists) used. All data were expressed as mean percentage change±SEM of renal vasoconstrictor responses elicited by all the frequencies (renal nerve stimulation) and all the doses (adrenergic agonists) and were compared between the phases (saline, low dose of chloroethylclonidine, and high dose of chloroethylclonidine-treated phases). In the renal vasoconstriction experiments, two-way ANOVA was used for the statistical analysis. For the analysis of the physiological and other data, one-way ANOVA was used followed by the *Bonferroni post-hoc* test (Superanova, Abacus, Barkley CA, USA). The differences between the means were considered significant at the 5% level. All physiological and biochemical data (body weight, 24 h water intake, 24 h urine output, plasma sodium, urinary sodium, fractional excretion of sodium, creatinine clearance, albumin excretion, glomerular filtration rate, and kidney index) were analyzed using one-way ANOVA followed by the *Bonferroni post-hoc* test[8,10,11,14].

## Results

**General observations** Renal failure was identified by increased plasma creatinine, reduced creatinine clearance, increased fractional excretion of sodium, reduced glomerular filtration rate, and diuresis in the cisplatin-treated renal failure rats (all $P<0.01$) as compared to the non-cisplatin-treated non-renal failure animals (Table 1). Moreover, there was a markedly increased ($P<0.01$) kidney index (percentage of kidney weight to body weight) and pronounced renal tubular damage in the renal failure as compared to the non-renal failure rats (Table 1; Figure 1). In the experimental early diabetic nephropathy rats, along with marked hyperglycemia, there was increased serum creatinine, creatinine clearance, urinary albumin excretion, glomerular filtration rate (all $P<0.01$), moderately increased fractional excretion of sodium ($P>0.05$), and an increased kidney index ($P<0.01$). Unlike the renal failure rats, there was no

**Table 1.** Renal functional and other parameters in rats with different pathophysiological states.

| Experimental group | Renal functional and other parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Kidney Index[#] | PNa (mmol/L) | PCr (mg/dL) | UNa (mmol/L) | UCr (mg/dL) | UAE (mg/min) | FENa (%) | CCr (mL/min per 100 g) | FBG (mmol/L) |
| Wistar Kyoto | 0.39±0.03 | 155.2±1.9 | 0.73±0.01 | 107.3±2.9 | 157.0±9.2 | Not Done | 0.3±0.01 | 1.0±0.03 | Not Done |
| Renal failure Wistar Kyoto | 0.76±0.01[c] | 113.3±6.0[c] | 2.79±0.46[c] | 131.3±9.1[c] | 41.8±5.9[c] | Not Done | 7.7±1.1[c] | 0.3±0.001[c] | Not Done |
| Spontaneously hypertensive rats | 0.41±0.02 | 141.6±1.9 | 0.83±0.07 | 128.2±3.0 | 153.2±7.0 | Not Done | 0.5±0.04 | 0.9±0.001 | Not Done |
| Renal failure Spontaneously hypertensive rats | 0.66±0.01[c] | 109.7±3.4[c] | 2.88±0.36[c] | 139.4±5.1[c] | 34.9±7.2[c] | Not Done | 10.0±0.78[c] | 0.3±0.001[c] | Not Done |
| Non-diabetic nephropathy rats | 0.33±0.02 | 149.2±5.2 | 0.77±0.04 | 124.6±1.8 | 144.1±3.6 | 0.69±0.02 | 0.44±0.03 | 0.9±0.002 | 5.8±0.7 |
| Experimental early diabetic nephropathy rats | 0.69±0.03[c] | 133.0±6.5[b] | 1.47±0.17[c] | 139.6±6.1[b] | 90.0±2.2[b] | 1.27±0.20[c] | 1.36±0.01[b] | 2.7±0.12[c] | 15.5±1.1[c] |

[b]$P<0.05$, [c]$P<0.01$ and the comparisons were made between Wistar Kyoto *vs* renal failure Wistar Kyoto; Spontaneously Hypertensive rats *vs* renal failure Spontaneously Hypertensive rats, non-diabetic nephropathy rats *vs* rats with experimental early diabetic nephropathy. All data were analyzed by two-way ANOVA followed by Bonferroni post-hoc test. Data presented here as mean±SEM ($n$=5–9).

[#]Kidney index was calculated as Kidney index=kidney weight/body weight (×100).

PNa=Plasma sodium; PCr=Plasma creatinine; UNa=Urinary sodium; UCr=Urinary creatinine; UAE=Urinary albumin; FENa=Fractional excretion of sodium; CCr=creatinine clearance and FBG=Fasting blood glucose

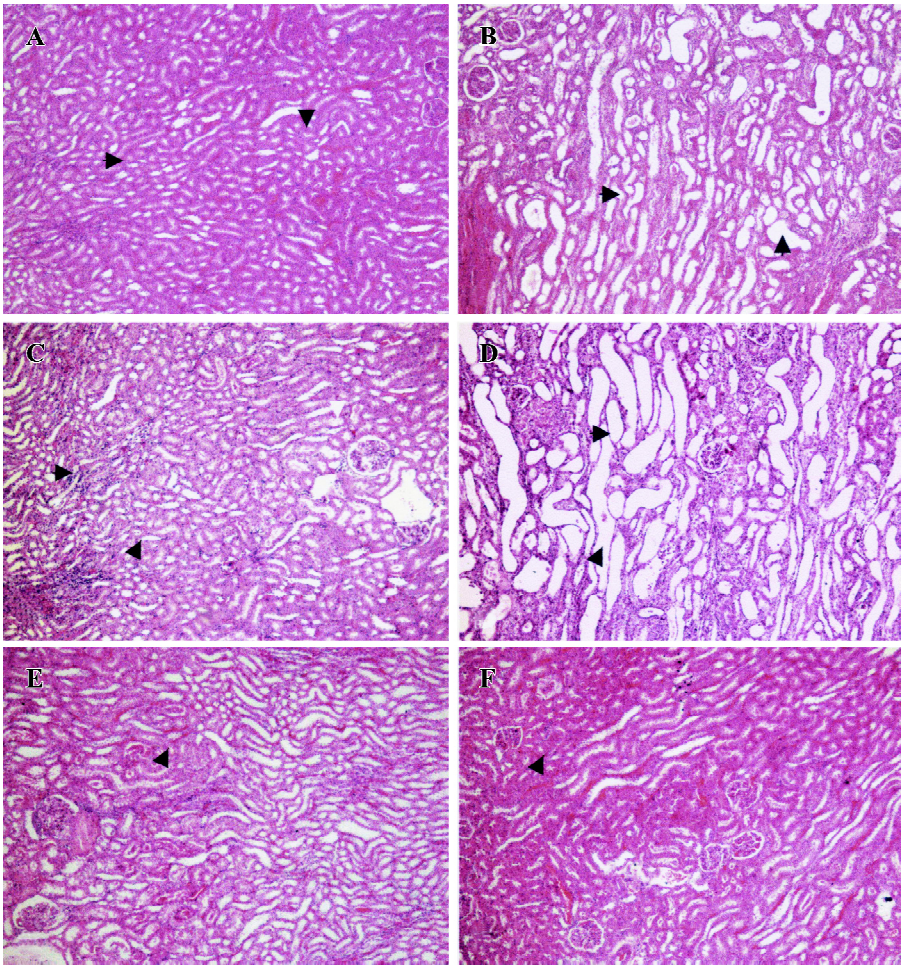


**Figure 1.** Light microscopy of renal tissue (5 μm) from Wistar Kyoto rats (A), renal failure Wistar Kyoto (B), spontaneously hypertensive rats (C), renal failure spontaneously hypertensive rats (D), control rats for experimental diabetic nephropathy (E) and experimental early diabetic nephropathy rats (F). Hematoxylin & eosin staining (×200). In renal failure rats either spontaneously hypertensive rats or Wistar Kyoto rats, a severe destruction of tubular structure was observed. However, in diabetic nephropathy rat there was no significant tubular damage observed. The arrows are indicating the areas with either normal tubular structure or damages.

adverse tubular structural change in these rats as compared to the non-diabetic nephropathy control rats (Table 1; Figure 1). The baseline mean arterial blood pressure and renal blood flow of all the experimental groups are presented in Table 2. In the renal failure rats, renal blood flow was significantly lower compared to the non-renal failure rats ($P<0.01$). In the experimental early diabetic nephropathy rats, renal blood flow was greater compared to the non-diabetic nephropathy rats. However, this change in renal blood flow did not attain statistical significance ($P>0.05$). In addition, the baseline mean arterial blood pressure of these rats (renal failure and experimental early diabetic nephropathy) was either lower or unaltered ($P>0.05$) in the diseased rats when compared with the non-renal failure or non-diabetic nephropathy rats.

**Renal vasoconstrictor responses**

Normal and renal failure Wistar Kyoto rats In the case of renal nerve stimulation, the administration of chloroethylclonidine produced an accentuation ($P<0.01$) of renal nerve stimulation-induced renal vasoconstrictor responses in the renal failure Wistar Kyoto rats; the magnitudes of these changes were dose-dependent (Figure 2). However, in normal Wistar Kyoto rats, chloroethylclonidine did not cause any marked alterations in such responses. The mixed adrenergic agonist noradrenaline-induced changes were also unaltered by either doses of chloroethylclonidine in these rats (Figure 2). In contrast, there was a marked dose-dependent accentuation ($P<0.01$) of the noradrenaline-induced renal vasoconstrictor responses in renal failure Wistar Kyoto rats (Figure 2). In renal failure Wistar Kyoto rats, phenylephrine and methoxamine-induced renal vasoconstrictor responses were attenuated by chloroethylclonidine ($P<0.01$). However, these responses in non-renal failure Wistar Kyoto rats were unaltered by chloroethylclonidine (Figure 2). The overall mean percentage changes in the renal blood flow of renal failure and non-renal failure Wistar Kyoto rats to different adrenergic stimuli are shown in Table 3.

Spontaneously hypertensive and renal failure spontaneously hypertensive rats Chloroethylclonidine pro-

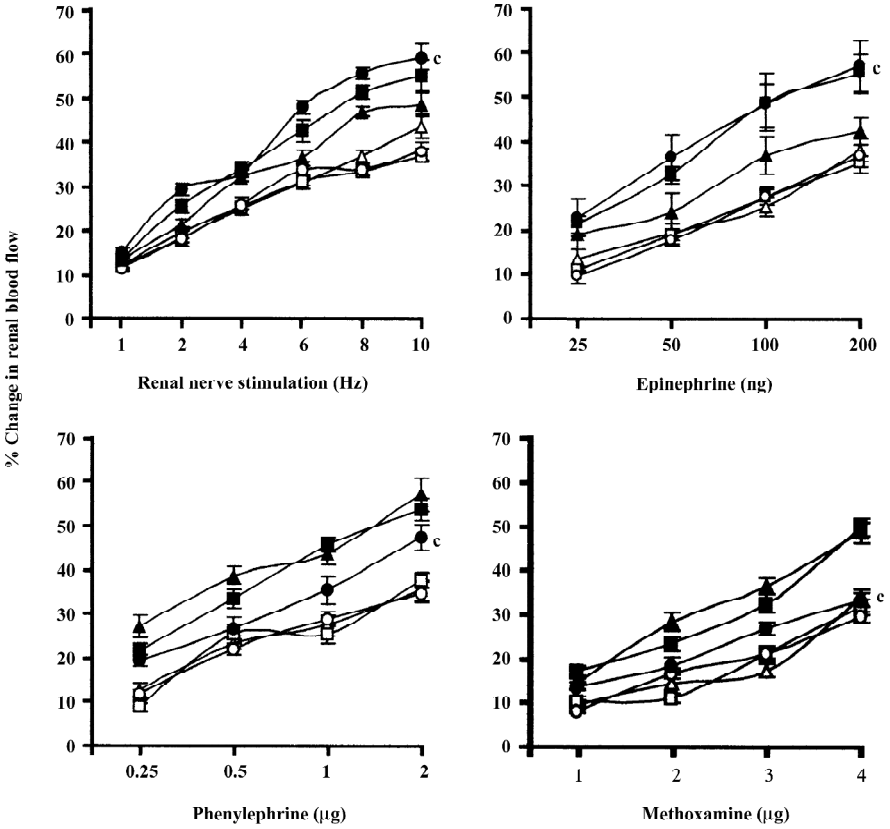


**Figure 2.** Adrenergically induced renal vasoconstrictor responses in the presence and absence of chloroethylclonidine in renal failure Wistar Kyoto (Saline ▲, Low dose ■, High dose ●) and non-renal failure Wistar Kyoto (Saline △, Low dose □, High dose ○) rats. $n$=5–9. Data presented as mean±SEM. $^cP<0.01$ (overall mean % change of renal blood flow in phase treated with saline *vs* phase treated with either low or high dose of chloroethylclonidine). Data were analyzed by two-way ANOVA followed by *Bonferroni post-hoc* test.

**Table 2.** Physiological and hemodynamic measurements in rats with different pathophysiological states.

| Experimental groups | Body weight (g) | Urine output (mL/24 h) | Water intake (mL/24 h) | Mean arterial pressure (mmHg) | Renal blood flow (mL·min$^{-1}$·kg$^{-1}$) | Glomerular filtration rate (mL/min) |
|---|---|---|---|---|---|---|
| Wistar Kyoto | 274±5.7 | 6.8±0.8 | 28.0±3.4 | 105.0±3.4 | 24.0±1.4 | 3.4±0.010 |
| Renal failure Wistar Kyoto | 264±6.3$^c$ | 26±0.9$^c$ | 46.9±2.7$^c$ | 94.0±6.2 | 10.0±0.2$^c$ | 0.4±0.001$^c$ |
| Spontaneously hypertensive rats | 284±8.1 | 7.1±0.7 | 31.6±3.4 | 142.0±1.6 | 18.0±0.8 | 3.0±0.007 |
| Renal failure spontaneously hypertensive rats | 269±6.1$^c$ | 32.2±1.1$^c$ | 53.0±2.4$^c$ | 139.2±4.2 | 6.0±0.4$^c$ | 0.2±0.001$^c$ |
| Control rats of experimental early diabetic nephropathy group | 278±6.3 | 7.4±0.8 | 23.0±1.7 | 126.0±2.2 | 17.0±0.2 | 3.1±0.004 |
| Experimental early diabetic nephropathy | 245±6.1$^c$ | 69.6±4.1$^c$ | 114.7±1.7$^c$ | 117.0±5.2 | 22.7±0.3 | 4.0±0.003$^b$ |

$^bP<0.05$, $^cP<0.01$ and the comparisons were made either between non-renal failure and renal failure or between non-diabetic nephropathy and early diabetic nephropathy rats. All data were analyzed by one-way ANOVA followed by *Bonferroni post-hoc* test. Data presented here as mean±SEM ($n$=5–9).

duced interesting results in renal failure spontaneously hypertensive rats with a marked attenuation (by the low dose of chloroethylclonidine) followed by accentuation (by the high dose of chloroethylclonidine) of the renal nerve stimu-

**Table 3.** The overall mean % changes in the renal blood flow to different stimuli in the absence and presence of low and high doses of chloroethylclonidine in different experimental groups of rat.

| Adrenergic stimuli | Dose of chloroethyl-clonidine (μg/kg) | % Change in renal blood flow | | | | | |
|---|---|---|---|---|---|---|---|
| | | Wistar-Kyoto rat (WKY) | | Spontaneously hypertensive rat (SHR) | | Diabetic nephropathy rat | |
| | | Non-renal failure WKY | Renal failure WKY | Non-renal failure SHR | Renal failure SHR | Non-diabetic nephropathy | Experimental early diabetic nephropathy |
| Renal nerve stimulation | 0 | 27.6±1.4 | 33.1±1.4 | 38.0±1.5 | 50.3±2.4 | 35.7±2.2 | 39.7±3.4 |
| (1–10 Hz) | 5 | 26.4±1.3 | 36.9±1.9[c] | 39.1±1.7 | 39.8±2.4[c] | 35.8±1.8 | 42.5±4.0 |
| | 10 | 26.9±1.3 | 40.0±1.5[c] | 38.4±1.3 | 59.1±1.9[c] | 35.4±1.8 | 45.8±2.4[c] |
| Noradrenaline | 0 | 24.1±2.3 | 30.6±3.1 | 30.2±1.0 | 43.8±2.0 | 31.9±2.2 | 30.1±3.6 |
| (25, 50, 100 & 200 ng) | 5 | 23.3±1.4 | 39.6±3.6[c] | 30.8±2.1 | 43.9±2.4 | 31.7±2.9 | 36.6±3.2 |
| | 10 | 23.0±1.7 | 41.2±5.0[c] | 29.8±2.3 | 43.3±3.9 | 31.5±1.5 | 39.5±1.9[c] |
| Phenylephrine | 0 | 24.8±1.9 | 41.7±2.7 | 26.2±1.0 | 37.9±2.0 | 26.1±2.6 | 31.3±2.9 |
| (0.25, 0.5, 1.0 & 2.0 mg) | 5 | 24.4±1.8 | 38.7±2.0 | 26.1±2.0 | 41.6±1.9 | 26.0±3.1 | 37.0±3.0 |
| | 10 | 24.3±1.6 | 32.3±2.5[c] | 26.5±2.9 | 43.2±1.8[c] | 27.0±3.0 | 40.0±3.7[c] |
| Methoxamine | 0 | 18.9±1.8 | 32.0±1.9 | 25.9±2.0 | 31.9±2.6 | 24.8±2.9 | 30.2±3.1 |
| (1, 2, 3 & 4 mg) | 5 | 18.8±1.3 | 30.8±1.6 | 24.3±2.2 | 38.0±2.1[c] | 24.3±2.5 | 34.8±3.1 |
| | 10 | 18.9±1.8 | 23.1±1.7[c] | 26.8±1.4 | 39.4±2.7[c] | 24.6±2.3 | 39.5±2.2[c] |

The comparisons were made either saline and low dose of chloroethylclonidine treated phases or between saline and high dose of chloroethylclonidine treated phases. All data were analyzed by two-way ANOVA followed by *Bonferroni post-hoc* test. Data presented here as mean±SEM ($n$=5–9). [c]$P$<0.01 *vs* Chloroethylclonidine 0 μg/kg.

lation-induced renal vasoconstrictor responses (all $P$< 0.01). However, in normal spontaneously hypertensive rats, chloroethylclonidine did not cause any meaningful alteration in the renal nerve stimulation-induced renal vasoconstrictor responses (Figure 3). In the case of noradrenaline-induced vasoconstrictor responses, chloroethyl clonidine at both doses did not canse any alteration ($P$>0.05) in either of the experimental groups. In the spontaneously hypertensive rats, neither dose of chloroethylclonidine showed any effect on the phenylephrine and methoxamine-induced renal vasoconstrictor responses ($P$>0.05). Interestingly, in the renal failure spontaneously hypertensive rats, chloroethyl-clonidine in its high dose caused enhancement of phenylephrine and methoxamine-induced renal vasoconstrictor responses ($P$< 0.01), while its low dose remained insensitive ($P$>0.05; Figure 3). The overall mean percentage changes in the renal blood flow of renal failure and non-renal failure spontaneously hypertensive rats to different adrenergic stimuli are shown in Table 3.

### Rats with experimental early diabetic nephropathy

In these group of rats chloroethylclonidine accentuated (all $P$<0.01) all the adrenergically-induced renal vasoconstrictor responses in the rats with experimental early diabetic nephropathy. However, in the non-diabetic nephropathy rats of this particular experimental group chloroethylclonidine did not cause any marked (all $P$<0.01) shift in these responses (Figure 4). The overall mean percentage changes in the renal blood flow of renal failure and non-diabetic nephropathy and experimental early diabetic nephropathy rats to different adrenergic stimuli are shown in Table 3.

## Discussion

This study, perhaps for the first time, shows the functional involvement of chloroethylclonidine sensitive $\alpha_{1B}$-adrenoceptors in modulating adrenergically-induced renal vasoconstrictions in renal failure, hypertensive renal failure, and early diabetic nephropathy rats. In the rats with normal renal functions, such functional contribution of this $\alpha_1$-adrenoceptor subtype was absent.

In the rats with renal impairment, the adrenergically-induced renal vasoconstrictions were either accentuated or attenuated by chloroethylclonidine, whereas, these responses in the non-renal failure and non-diabetic nephropathy rats remain unchanged. A similar set of observations has been made in several earlier studies on rats with different pathological states, particularly in some types of experimental hypertension, heart failure, diabetes, and also in a combined state of hypertension and diabetes[10,11,14]. Most importantly,

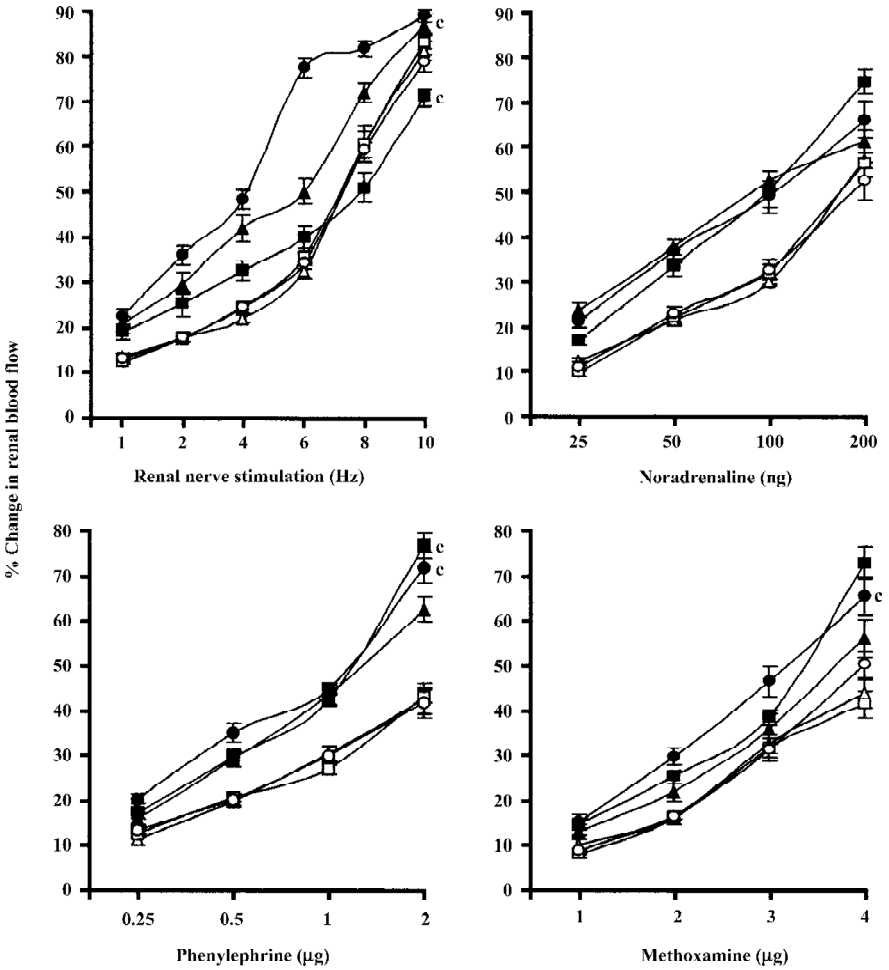


**Figure 3.** Adrenergically induced renal vasoconstrictor responses in the presence and absence of chloroethylclonidine in renal failure spontaneously hypertensive (Saline ▲, Low dose ■, High dose ●) and non-renal failure spontaneously hypertensive (Saline △, Low dose □, High dose ○) rats. Data presented as mean±SEM ($n$=5–9). $^cP$ <0.01 (overall mean % change of renal blood flow in phase treated with saline *vs* phase treated with either low or high dose of chloroethyl-clonidine). Data were analyzed by two-way ANOVA followed by *Bonferroni post-hoc* test.

these studies have indicated a possible minor functional involvement of $\alpha_{1B}$-adrenoceptors in the adrenergically-induced renal vasoconstrictions in these rats. In normal rats, however, no such involvement of $\alpha_{1B}$-adrenoceptors in modulating renal vasoconstrictions has been reported. In this context, the present study investigated whether a similar phenomenon could exist in some important pathological conditions characterized with impaired renal functions and provides further support to the earlier stated view on the interesting functional characteristics of $\alpha_{1B}$-adrenoceptors in renal vasculature.

The present study showed that in the rats with renal impairment, chloroethylclonidine accentuated the adrenergically-induced renal vasoconstrictor responses, with some exceptions in renal failure Wistar Kyoto rats where chloroethylclonidine attenuated the phenylephrine and methoxamine induced responses. In general, however, there was an enhancement of the renal vasoconstrictor responses elicited by renal nerve stimulation and noradrenaline (renal failure Wistar Kyoto rats); by renal nerve stimulation, phenylephrine, and methoxamine (renal failure spontaneously hypertensive rats), and also by renal nerve stimulation and all the adrenergic agonists (rats with experimental early diabetic nephropathy) in the presence of chloroethylclonidine.

It has been shown that in some vasculature, noradrenaline, which is released due to the stimulation of renal nerves, caused $\alpha_{1A}$-adrenoceptor subtype-mediated vasoconstriction[16,34]. Like endogenously-released noradrenaline, exogenously administered noradrenaline is also reported to exert its action through $\alpha_{1A}$-adrenoceptor subtypes with little or no evidence for the involvement of $\alpha_{1B}$-, $\alpha_{1D}$-, and even $\alpha_2$-adrenoceptors[16]. As an apparent difference of some of these findings, there are reports on the involvement of $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptors in mediating exogenously administered and endogenously available noradrenaline in *in vitro* experiments[15,16,35]. However, as asserted in some earlier reported findings, in the presence of chloroethylclonidine and enhanced sympathetic activity, any such involvement of $\alpha_{1B}$-

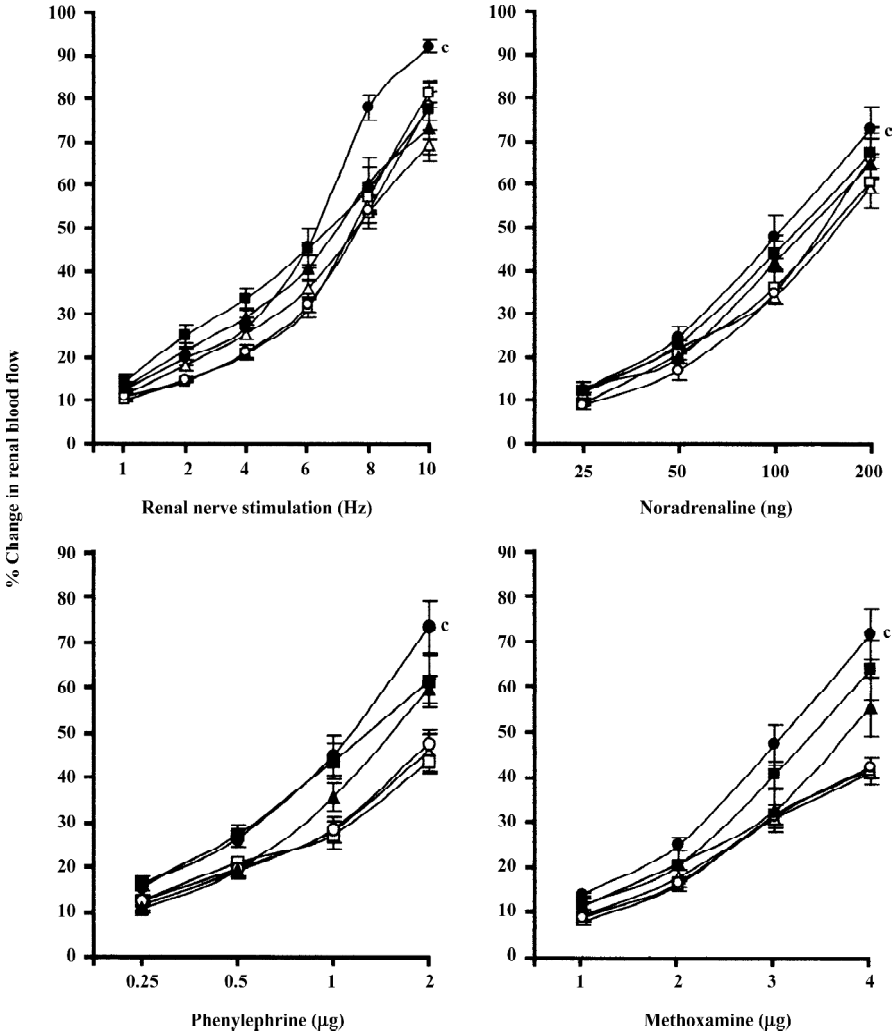


**Figure 4.** Adrenergically induced renal vasoconstrictor responses in the presence and absence of chloroethylclonidine in experimental early diabetic nephropathy (Saline ▲, Low dose ■, High dose ●) and non-diabetic nephropathy (Saline △, Low dose □, High dose ○) rats. $n$=5–9. Data presented as mean±SEM. $^cP<0.01$ (overall mean % change of renal blood flow in phase treated with saline *vs* phase treated with either low or high dose of chloroethylclonidine). Data were analyzed by two-way ANOVA followed by *Bonferroni post-hoc* test.

and $\alpha_{1D}$-adrenoceptors in mediating renal vasoconstriction will be abolished or impeded. It is stated that chloroethylclonidine has selectivity for $\alpha_1$-adrenoceptors in the order of $\alpha_{1B} > \alpha_{1D} >> \alpha_{1A}$, and that the $\alpha_{1A}$-adrenoceptor is insensitive to chloroethylclonidine, hence, the $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptor subtypes will preferentially be inactivated by chloroethylclonidine[36–40] leaving the $\alpha_{1A}$-adrenoceptors to be acted upon by the adrenergic stimuli, leading to vasoconstrictions. Apart from the possible inactivation by chloroethylclonidine, the suggested inability of the $\alpha_{1D}$-adrenoceptor subtypes to mediate vasoconstrictions in these pathological states that are characterized with enhanced sympathetic activity, can further be explained based on the observation that the $\alpha_{1D}$-adrenoceptor subtypes are phosphorylated in the face of enhanced sympathetic activity[4,13,41]. Indeed, the augmented renal vasoconstrictor response is reported in several pathophysiological states (spontaneously hypertensive rats, diabetic hypertensive rats, renal failure rats, and 2K1C Goldblatt hypertensive rats), as reported earlier[10,11,14,17].

With this background, it can be suggested that in the presence of chloroethylclonidine, both renal nerve stimulation and noradrenaline-induced renal vasoconstrictions was mediated by $\alpha_{1A}$-adrenoceptors in the rats with renal impairment. This view can further be explained based on an earlier report that in these rats, chloroethylclonidine blocked $\alpha_{1B}$-adrenoceptor subtypes and caused $\alpha_{1A}$-adrenoceptor subtype-mediated renal vasoconstrictor responses to be accentuated by the endogenous or exogenously administered noradrenaline. It can also be suggested that in these rats there could be an upregulation of $\alpha_{1A}$-adrenoceptors or involvement of presynaptic $\alpha_{1B}$-adrenoceptors in determining renal vasoconstriction. In the later case, it can be suggested that when these receptors are blocked by chloroethylclonidine,

the presynaptic autoinhibitory feedback is removed and allow more noradrenaline to be released, which consequently resulted in a larger post-synaptic response[22]. Another possible explanation of these observations in the rats with renal impairment could be the downregulation of certain $\alpha_1$-adrenoceptors in the renal vasculature. In support of this view, it is reported that in diabetes and hypertension, the $\alpha_{1B}$-adrenoceptors could be downregulated by the adrenergic nerves leaving postsynaptic receptors, like the $\alpha_{1A}$-adrenoceptor (the predominant type in renal vasculature), to be enhanced in order to maintain the effectiveness of the $\alpha_1$-adrenergic nervous system[32,4].

Chloroethylclonidine showed a similar accentuating effect on the phenylephrine and methoxamine-mediated responses in the renal failure spontaneously hypertensive and experimental early diabetic nephropathy rats, while in the non-renal failure and non-diabetic nephropathy rats it was insensitive. These accentuations of renal vasoconstrictions caused by phenylephrine (selective to the $\alpha_1$-adrenoceptor subtypes) and methoxamine (selective to the $\alpha_{1A}$-and $\alpha_{1D}$-adrenoceptor subtypes) can be explained in light of our discussion on the accentuation of noradrenaline and renal nerve stimulation-mediated responses in the presence of chloroethylclonidine.

In renal failure spontaneously hypertensive rats, it was further observed a biphasic action of chloroethylclonidine on the renal nerve stimulation induced changes. It is reported that there could be a crosstalk relationship between the $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes in terms of inhibition of $\alpha_{1A}$-adrenoceptor activities by $\alpha_{1B}$-adrenoceptor subtypes[43]. This phenomenon could help us to explain the observed biphasic action of chloroethylclonidine in renal nerve stimulation-induced renal vasoconstrictor responses in renal failure spontaneously hypertensive rats.

In the case of low dose of chloroethylclonidine, the puzzling attenuation of the renal nerve-induced vasoconstrictions in renal failure spontaneously hypertensive rats could be attributed to the blockade of the unsaturated presynaptic $\alpha_{1B}$-adrenoceptors, and it is widely reported that chloroethylclonidine can act presynaptically. In explaining the accentuation the vasoconstrictor responses in these rats by a higher dose of chloroethylclonidine, it is possible that the occupation of the $\alpha_{1B}$-adrenoceptors leads to an alteration of the properties of $\alpha_{1A}$-adrenoceptors so that the normal agonist and antagonist interaction can not occur. In this situation, it could be a possibility that the blockade of $\alpha_{1B}$-adrenoceptors by chloroethylclonidine would enhance the sensitivity of the remaining $\alpha_1$-adrenoceptors ( $\alpha_{1A}$-and $\alpha_{1D}$-adrenoceptors) so that a potentiation of renal vasoconstrictor responses occurs. Another possibility is that, in this particular pathological state with renal failure and hypertension, there might be an activation of spare receptors due to the blockade of the $\alpha_{1B}$-adrenoceptors[17,10,14,44].

Together, these data lead us to suggest that in renal failure Wistar Kyoto and spontaneously hypertensive rats, and also in the rats with experimental early diabetic nephropathy, there was a functional involvement of the $\alpha_{1B}$-adrenoceptors in modulating adrenergically-induced renal vasoconstric-tions. The results obtained also lead us to suggest that there might be a complex interaction between the chloroethylclonidine sensitive $\alpha_1$-adrenoceptor subtypes. In non-renal failure and non-diabetic nephropathy rats, the functional involvement of the $\alpha_{1B}$-adrenoceptor subtypes was absent.

## References


1 DiBona GF, Sawin LL. Role of renal $\alpha_2$-adrenergic receptors in spontaneously hypertensive rats. Hypertension 1987; 9: 41–81.

2 Sattar MA, Johns EJ. Evidence for an alpha 1-adrenoceptor subtypes mediating adrenergic vasoconstriction in Wistar normotensive and stroke prone hypertensive rats. J Cardiovasc Pharmacol 1994; 23: 232–9.

3 Yang M, Reese J, Cotecchia S, Michel MC. Murine alpha1-adrenoceptor subtypes. I. Radioligand binding studies. J Pharmacol Exp Ther 1998; 286: 841–7.

4 García-Sáinz JA, Vazuez-Prado J, Medina LC. $\alpha_1$-adrenoceptors: functions and phosphorylation. Eur J Pharmacol 2000; 389: 1–12.

5 Guimaraes S, Moura D. Vascular adrenoceptors: an update. Pharmacol Rev 2001; 53: 319–56.

6 Williams TJ, Clarke DE. Characterization of $\alpha_1$-adrenoceptors mediating vasoconstriction to noradrenaline and nerve stimulation in the isolated perfused mesentery of rat. Br J Pharmacol 1995; 114: 531–6.

7 Salomonsson M, Brannstrom K, Arendshorst WJ. $\alpha_1$-adrenoceptor subtypes in rat renal resistance vessels: *in vivo* and *in vitro* studies. Am J Physiol 2000; 278: F138–47.

8 Hirasawa A, Sugawara T, Awaji T, Tsumaya K, Ito H, Tsujimoto G. Subtype-specific differences in subcellular localization of alpha1-adrenoceptors: chlorethylclonidine preferentially alkylates the accessible cell surface alpha1-adrenoceptors irrespective of the subtype. Mol Pharmacol 1997; 52: 764–70.

9 Walsh MP. Regulation of vascular smooth muscle tone. Can J Physiol Pharmacol 1994; 72: 919–36.

10 Abdul Sattar M, Johns EJ. Alpha 1-adrenoceptor subtypes mediating adrenergic vasoconstriction in kidney, one-clip Goldblatt and deoxycorticosterone acetate-salt hypertensive rats. J Cardiovasc Pharmacol 1994; 24: 420–8.

11 Yatsu T, Aoki M, Inagaki O. Preventive effect of zelandopam, a dopamine D1 receptor agonist, on cisplatin-induced acute renal failure in rats. Eur J Pharmacol 2003; 461: 191–5.

12 Villalobos-Molina R, López-Guerrero JJ, Ibarra M. Alpha$_{1D}$- and alpha$_{1A}$-adrenoceptors mediate contraction of rat renal artery. Eur J Pharmacol 1997; 322: 225–7.

13 Arévalo-León LE, Gallardo-Ortíz IA, Urquiza-Marín H, Villalobos-Molina R. Evidence for the role of alpha$_{1D}$- and $\alpha_{1A}$-adrenoceptors in contraction of rat masentric artery. Vascular Pharmacol 2003; 40: 91–6.
14 Armenia A, Munavvar AS, Abdullah NA, Helmi A, Johns EJ. The contribution of adrenoceptor subtypes in the renal vasculature of diabetic spontaneously hypertensive rats. Br J Pharmacol 2004; 142: 719–26.
15 Jahnichen S, Eltze M, Pertz HH. Evidence that alpha (1B)-adrenoceptors are involved in noradrenaline-induced contractions of rat tail artery. Eur J Pharmacol 2004; 488: 157–67.
16 Zacharia J, Hillar C, MacDonald A. $\alpha_1$-adrenoceptor subtypes involved in vasoconstrictor responses to exogenous and neurally released in rat femoral resistance arteries. Br J Pharmacol 2004; 141: 915–24.
17 Khan AH, Sattar MA, Abdullah NA, Johns EJ. Impact of renal failure on the $\alpha_1$-adrenoceptor subtype mediating vasoconstriction in the kidney of the rat. Eur J Pharmacol 2007; 569: 110–8.
18 Abbas SA, Munavvar AS, Abdullah NA, Johns EJ. Involvement of $\alpha_1$-adrenoceptor subtypes in the cardiac failure in spontaneously hypertensive rats. J Basic Applied Sci 2006; 2: 59–69.
19 Stassen FR, Willemsen MJ, Janssen GM, DeMey JG. Alpha 1-adrenoceptor subtypes in rat aorta and mesenteric small arteries are preserved during left ventricular dysfunction post-myocardial infarction. Cardiovasc Res 1997; 33: 706–13.
20 Kang DG, Lee AS, Mun YJ, Woo WH, Kim YC, Sohn EJ, *et al*. Butein ameliorates renal concentrating ability in cisplatin-induced acute renal failure in rats. Biol Pharm Bull 2004; 27: 366–70.
21 Yatsu T, Aoki M, Inagaki O. Preventive effect of zelandopam, a dopamine D1 receptor agonist, on cisplatin-induced acute renal failure in rats. Eur J Pharmacol 2003; 461: 191–5.
22 Shimeda Y, Hirotani Y, Akimoto Y, Shindou K, Ijiri Y, Nishihori T, *et al*. Protective effects of capsaicin against cisplatin-induced nephrotoxicity in rats. Biol Pharm Bull 2005; 28: 1635–8.
23 Saad SY, Al-Rikabi AC. Protection effects of taurine supplementation against cisplatin-induced nephrotoxicity in rats. Chemotherapy 2002; 48: 42–8.
24 Yin XX, Zhang YD, Shen JP, Wu HW, Zhu X, Li LM, *et al*. Protective effects of bendazac lysine on early experimental diabetic nephropathy in rats. Acta Pharmacol Sin 2005; 26: 721–8.
25 Saad SY, Arafah MM, Najjar TA. Effects of mycophenolate mofetil on cisplatin-induced renal dysfunction in rats. Cancer Chemother Pharmacol 2007; 59: 455–60.
26 Cooper ME, Allen TJ, Jerums G, Doyle AE. Accelerated progression of diabetic nephropathy in the spontaneously hypertensive streptozo-tocin diabetic rat. Clin Exp Pharmacol Physiol 1986; 13: 655–62.
27 Usui H, Shikata K, Matsuda M, Okada S, Ogawa D, Yamashita T, *et al*. HMG-CoA reductase inhibitor ameliorates diabetic nephropathy by its pleiotropic effects in rats. Nephrol Dial Transplant 2003; 18: 265–72.
28 Yin X, Zhang Y, Wu H, Zhu X, Zheng X, Jiang S, *et al*. Protective effects of *Astragalus saponin* I on early stage of diabetic nephropathyin rats. J Pharmacol Sci 2004; 95: 256–66.
29 Cooper ME. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia 2001; 44: 1957–72.
30 Nakano R, Kurosaki E, Shimaya A, Kajikawa S, Shibasaki M. YM440, a novel hypoglycemic agent, protects against nephropathy in Zucker fatty rats via plasma triglyceride reduction. Eur J Pharmacol 2006; 549: 185–91.
31 Cruzado JM, Lloberas N, Torras J, Riera M, Fillat C, Herrero-Fresneda I, *et al*. Regression of advanced diabetic nephropathy by hepatocyte growth factor gene therapy in rats. Diabetes 2004; 53: 1119–27.
32 Ibarra M, Pardo JP, López-Guerrero JJ, Villalobos-Molina R. Differential response to chloroethylclonidine in blood vessels of normotensive and spontaneously hypertensive rats: role of alpha 1D- and alpha 1A-adrenoceptors in contraction. Br J Pharmacol 2000; 129: 653–60.
33 Perez DM, Piascik MT, Malik N, Gaivin R, Graham RM. Cloning, expression, and tissue distribution of the rat homolog of the bovine alpha 1C-adrenergic receptor provide evidence for its classification as the alpha 1A subtype. Mol Pharmacol 1994; 46: 823–31.
34 Jahnichen S, Eltze M, Pertz HH. Evidence that alpha (1B)-adrenoceptors are involved in noradrenaline-induced contractions of rat tail artery. Eur J Pharmacol 2004; 488: 157–67.
35 Williams TJ, Clarke DE. Characterization of $\alpha_1$-adrenoceptors mediating vasoconstriction to noradrenaline and nerve stimulation in the isolated perfused mesentery of rat. Br J Pharmacol 1995; 114: 531–6.
36 Leclerc G, Rouot B, Schwartz J, Velly J, Wermuth CG. Studies on some para-substituted clonidine derivatives that exhibit an alpha-adrenoceptor stimulant activity. Br J Pharmacol 1980; 71: 5–9.
37 Docherty JR, O'Rourke M. The $\alpha$-adrenoceptor-mediated actions of chloroethylclonidine. Gen Pharmacol 1997; 28: 97–101.
38 Hirasawa A, Sugawara T, Awaji T, Tsumaya K, Ito H, Tsujimoto G. Subtype-specific differences in subcellular localization of alpha1-adrenoceptors: chlorethylclonidine preferentially alkylates the accessible cell surface alpha1-adrenoceptors irrespective of the subtype. Mol Pharmacol 1997; 52: 764–70.
39 Yang M, Reese J, Cotecchia S, Michel MC. Murine alpha1-adrenoceptor subtypes. I. Radioligand binding studies. J Pharmacol Exp Ther 1998; 286: 841–7.
40 Ibarra M, Lopez-Guerrero JJ, Villalobos-Molina R. The influence of chloroethylclonidine-induced contraction in isolated arteries of Wistar Kyoto rats: alpha$_{1D}$- and alpha$_{1A}$-adrenoceptors, protein kinase C, and calcium influx. Arch Med Res 2001; 32: 258–62.
41 García-Sáinz JA, Vazuez-Prado J, Medina LC. $\alpha_1$-adrenoceptors: functions and phosphorylation. Eur J Pharmacol 2000; 389: 1–12.
42 Li Z, Silver WP, Koman LA, Strandhoy JW, Rosencrance E, Gordon S, *et al*. Role of alpha-1 adrenoceptor subtypes mediating constriction of the rabbit ear thermoregulatory microvasculature. J Orthop Res 2000; 18: 156–63.
43 Kahan T. Prejunctional adrenergic receptors and sympathetic neurotransmission: studies in canine skeletal muscle vasculature *in situ*. Acta Physiol Scand 1987; 560: 1–38.
44 Piascik MT, SparkS MS, Pruitt TA, Soltis EE. Evidence for a complex interaction between the subtypes of the $\alpha_1$-adrenoceptor. Eur J Pharmacol 1991; 199: 279–89.