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ROLE OF α_1 ADRENOCEPTOR SUBTYPES IN RENAL HAEMODYNAMICS IN HEART FAILURE AND DIABETIC SD RATS

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ABSTRACT

Diabetes has been declared as one of the major global health hazards by the WHO and it leads to renal, cardiac and nervous tissue complications. Therefore, the present study was designed to examine the specific subclass of α adrenoceptors that are involved in the regulation of renal haemodynamics in diabetes and cardiac failure induced SD rats. Diabetes was induced by a single dose of streptozotocin (55mg/kg IP). Cardiac failure was induced by the combined treatment of caffeine and isoprenaline for seven days. On day eight the animals were anaesthetized by pentobarbitone sodium and the left kidney was exposed. The renal artery was cleared and electromagnetic flow probe was placed on it for renal blood flow (RBF) measurement. The left iliac artery was cannulated for the infusion of saline and all drugs. The renal nerves were stimulated by bipolar electrodes. The reduction in RBF to electrical nerve stimulation (1-10 Hz), bolus doses of noradrenaline (25-200 ng), phenylephrine (0.25-2.0 μ/kg) and methoxamine (1-4 μ/kg were determined before and after bolus doses of amlodipine (200 and 400 μ/kg), 5- methylurapidil (5 and 10 μ/kg), chloroethylclonidine (5 and 10 μ/kg) and BMY7378 (100 and 200 μ/kg). The results obtained indicated that the renal vasoconstrictor responses in this model were attenuated mainly by amlodipine, 5 methylurapidil and BMY7378 but not by chloroethylclonidine. The findings from this study suggest that α_{1A} and α_{1D} - adrenoceptors mediate the adrenergically induced renal vasoconstrictor responses in cardiac failure SD rats with diabetes.

Keywords: α_1 adrenoceptors, SD, cardiac failure.

INTRODUCTION

Adrenoceptors mediate the central and peripheral actions of noradrenaline and adrenaline. Adrenoceptors are found in most of the peripheral tissues and neurons within the central nervous system. These adrenoceptors mediate a variety of functions such as blood pressure and myocardial contractile rate and force. In the kidney the α-adrenoceptors modulate renal blood flow, electrolyte balance and metabolism. The α_1-adrenoceptors control blood flow and increase sodium reabsorption and gluconeogenesis. α_{1A} and α_{1D}- adrenoceptor subtypes primarily mediate adrenergically induced constriction of renal vasculature.

α_1-Adrenergic receptors are important mediators, which are involved in the regulation of vascular activity, and vascular smooth muscles to cause contraction response induced by norepinephrine (NE). α_1-Adrenoceptors are subdivided into three subtypes (α_{1A}, α_{1B}, and α_{1D}) by pharmacological techniques with subtype-selective antagonists and molecular biological techniques (Han *et al*., 2003). The corresponding cDNA coding for these receptors had been cloned by means of molecular biological methods. The distribution of α_1-Adrenoceptors and α_1-Adrenoceptors subtypes has been extensively characterized in rat and rabbit vessels using functional studies and molecular biological methods (Dong *et al.*, 1996; Han *et al.,* 1999). There were more α_{1A}-adrenoceptor subtypes in human renal cortex than the other subtypes. Expression of the three α_1- adrenoceptor subtypes mRNAs were confirmed in the arteries of the renal cortex, but among the three subtypes the α_{1B} was less apparent by *in situ* hybridization. Intense α_1-mRNA staining was apparent especially in the smooth muscles of the arterial walls of the kidney. In both proximal and distal renal tubules, each of the α_1-mRNAs were less marked in cytoplasm than in the arteries. The role of α_1-adrenoceptors in mediating the rat renal vasoconstriction has been well documented by the previous studies (Han and Minneman, 1990 and Sattar and Johns, 1996) and it has been discovered that the adrenergically induced vasoconstriction was primarily mediated by the α_{1A}-adrenoceptors in normotensive (Sattar and Johns, 1994a; Blue *et al*., 1995), SHR, SPSHR and 2 kidney one clip Goldblatt and DOCA-salt hypertensive rats (Sattar and Johns, 1994a and b). Kong *et al*. (1994) also studied that the sympathetic nerve stimulation of the perfused kidney provokes vasoconstriction which is extremely sensitive to low concentration of α_{1A}- adrenoceptor antagonist but not CEC.

However, there is little or no information available regarding the contribution of α_1-adrenoceptor subtypes in mediating renal vasoconstriction in cardiac failure and its combination with diabetes in Sprague Dawley rats. This study is one the first to examine the contribution of α_1-adrenoceptor subtype(s) in mediating the adrenergically induced renal vasoconstrictor responses in cardiac failure and diabetes induced Sprague Dawley rats.

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Table 1. Base line values of MAP in amlodipine, 5 methylurapidil, chloroethylclonidine, and BMY7378 treated cardiac failure induced diabetic and non-diabetic SD rats.

Antagonists	Doses (μg/kg)	SD (MAP in mm Hg)	
		Normal	Diabetic
Amlodipine	0	135±3	130±4
	100	130±4	125±3
	200	125±5	120±4
Chloroethylclonidine	0	136±4	135±3
	5	130±3	130±2
	10	128±3	128±3
5-methylurapidil	0	138±4	133±4
	5	135±3	130±3
	10	130±4	125±4
BMY7378	0	130±2	125±5
	100	125±4	120±3
	200	117±3	116±3

Table 2. Base line values of renal blood flow in amlodipine, 5 methylurapidil, chloroethylclonidine, and BMY7378 treated cardiac failure induced diabetic and non-diabetic SD rats.

Antagonists	Doses (μg/kg)	Dynamic renal blood flow ml/min/kg	
		Normal	Diabetic
Amlodipine	0	15.20 ± 0.64	14.51 ± 1.21
	100	15.41 ± 0.55	14.42 ± 1.31
	200	15.33 ± 0.44	14.53 ± 1.42
Chloroethylclonidine	0	13.00 ± 0.64	14.21 ± 0.08
	5	13.21 ± 0.51	14.12 ± 0.91
	10	13.3 ± 0.31	14.21 ± 0.88
5 methylurapidil	0	12.9 ± 1.17	13.0 ± 1.51
	5	12.7 ± 1.3	13.21 ± 1.2
	10	12.6 ± 1.6	13.31 ± 1.32
BMY7378	0	14.2 ± 0.54	13.21 ± 1.10
	100	14.0 ± 0.32	13.11 ± 1.22
	200	14.1 ± 0.51	13.23 ± 1.31

MATERIALS AND METHODS

Male Sprague Dawley rats (300-325g) were used in this study. Rats were bred in and obtained from Universiti Sains Malaysia's animal house. Diabetes and cardiac failure was induced in those rats. Diabetes was induced by the single dose of streptozotocin (55mg/kg IP). Whereas, cardiac failure was induced by the combined treatment of caffeine (40mg/kg) and isoprenaline (5mg/kg) for seven days. On day eight the rats were used for acute study. The animal was anaesthetised with pentobarbitone sodium (60mg/kg IP). The right carotid artery was cannulated for blood pressure measurements (Statham Grass model 79E polygraph). The left jugular vein was cannulated to allow infusion of saline and maintenance dose of anaesthetic (12.5mg/kg/h) which was given at the rate of 6 ml/h throughout the experiment.

The left kidney was exposed by an abdominal mid-line incision. The left iliac artery was cannulated with PP50, with a beveled tip which was advanced into the abdominal aorta, such that it lay at the level of the renal artery to enable the infusion of saline and administration of all drugs close renal arterially. The renal artery was cleared and an electromagnetic flow probe (EP 100 series) was placed on it. The probe was connected to a Square-Wave Electromagnetic Flow meter (Model FM 501 King, NC, Carolina Medical Electronic Inc) for renal blood flow measurements. The left renal nerves, passing from the coeliac and aortico-renal ganglia, were identified, cleaned of peritoneal lining, dissected for a short length to enable them to be placed on a bipolar silver stimulating electrode and sectioned. The functionality of the renal nerves was established before and after sectioning by stimulating the bundle for 15-30 seconds and observing that a blanching of the kidney occurred.

Table 3. Base line values of MAP in amlodipine, 5 methylurapidil, chloroethylclonidine, and BMY7378 treated cardiac failure induced diabetic and non-diabetic SD rats.

Antagonists	Doses (μg/kg)	SD (MAP in mm Hg)	
		Normal	Diabetic
Amlodipine	0	135 ± 3	130 ± 4
	100	130 ± 4	125 ± 3
	200	125 ± 5	120 ± 4
Chloroethylclonidine	0	136 ± 4	135 ± 3
	5	130 ± 3	130 ± 2
	10	128 ± 3	128 ± 3
5-methylurapidil	0	138 ± 4	133 ± 4
	5	135 ± 3	130 ± 3
	10	130 ± 4	125 ± 4
BMY7378	0	130 ± 2	125 ± 5
	100	125 ± 4	120 ± 3
	200	117 ± 3	116 ± 3

Table 4. Base line values of renal blood flow in amlodipine, 5 methylurapidil, chlorethylclonidine, and BMY7378 treated cardiac failure induced diabetic and non-diabetic SD rats.

Antagonists	Doses (μg/kg)	Dynamic renal blood flow ml/min/kg	
		Normal	Diabetic
Amlodipine	0	15.20 ± 0.64	14.51 ± 1.21
	100	15.41 ± 0.55	14.42 ± 1.31
	200	15.33 ± 0.44	14.53 ± 1.42
Chloroethylclonidine	0	13.00 ± 0.64	14.21 ± 0.08
	5	13.21 ± 0.51	14.12 ± 0.91
	10	13.3 ± 0.31	14.21 ± 0.88
5 methylurapidil	0	12.9 ± 1.17	13.0 ± 1.51
	5	12.7 ± 1.3	13.21 ± 1.2
	10	12.6 ± 1.6	13.31 ± 1.32
BMY7378	0	14.2 ± 0.54	13.21 ± 1.10
	100	14.0 ± 0.32	13.11 ± 1.22
	200	14.1 ± 0.51	13.23 ± 1.31

Upon completion of surgery, 2ml of saline was given intravenously as a primer and an infusion of saline containing sodium pentobarbitone 12.5mg/kg/h was started at a rate of 6ml/h via the close renal arterial cannula. The animals were given one hour to stabilize before the commencement of experiment.

Experimental protocols

Eight groups of rats were studied, groups 1-4 and 5-8 were non-diabetic SD and diabetic SD. Renal vasoconstrictor responses were determined by:

1. Electrical renal nerve stimulations
2. Different doses of agonists (NA, PE and ME)

The reduction in RBF to electrical nerve stimulation (at 1, 2, 4, 6, 8 and 10 Hz at 15 V and 2ms), bolus doses of noradrenaline (25, 50, 100 and 200 ng), phenylephrine (0.25, 0.5, 1.0 and 2.0 μg) and methoxamine (1, 2, 3 and 4 μg) were determined before and after bolus doses of antagonists i.e amlodipine (200 and 400 μg/kg plus 50 and 100 μg/kg/h), 5 methylurapidil (5 and 10 μg/kg plus 1.25 and 2.5 μg/kg/h), chloroethylclonidine (5 and 10 μg/kg plus 1.25 and 2.5 μg/kg/h) and BMY7378 (100 and 200 μg/kg plus 25 and 50 μg/kg/h).

Renal nerve stimulations

The renal nerves were stimulated at a number of different frequencies, 1, 2, 4, 6, 8 and 10 Hz, at 0.2 ms duration and 15 V for periods of 20 seconds and in a sequence of ascending followed by descending frequencies such that two responses at each frequency was obtained.

Different doses of agonists (NA, PE and ME)

Bolus doses of NA, 25, 50, 100, and 200 ng, PE, 0.25, 0.5, 1.0 and 2.0 µg and ME, 1, 2, 3, and 4 µg were given close renal arterially in increasing and decreasing doses such that two responses per dose of the agonist were obtained. The determination of control renal vasoconstrictor responses to renal nerve stimulation, NA, PE and ME were carried out 15 minutes after the administration of the bolus dose of the vehicle. When the control responses had been recorded, the first dose of antagonist was given immediately followed by the respective infusion and 15 minutes later the sequence of nerve stimulation and agonist administration was repeated. The second dose of the antagonist was then given and the supporting infusion started immediately and after 15 minutes the sequence of renal vasoconstrictor responses induced by renal nerve stimulation and the agonists determined. A renal vasoconstrictor response was taken as the peak reduction in the renal blood flow to either direct renal nerve stimulation or the agonist and the average of the two values obtained were used for the calculation. The renal blood flow was allowed to return to normal (baseline) level before commencing with subsequent renal nerve stimulation or of agonist administration. The renal vasoconstrictor responses obtained after bolus injection of vehicle served as controls for each experimental group. The doses of agonists and antagonists were adopted from the previous and our own preliminary studies (Sattar and Johns, 1994a and Armenia, 2000).

Mean Arterial Pressure (MAP)

The mean arterial pressure was monitored continuously throughout the experiment, but only that at the beginning of each part of vasoconstrictor response was taken as the base line pressure.

Preparation of Drugs

CEC, AMP and BMY7378 were dissolved in normal saline, whereas 5MeU was dissolved in 10 mmol lactic acid in normal saline (Sattar and Johns, 1996). All the agonists and antagonists were prepared fresh before starting the experiment.

Statistical analysis

The renal vasoconstrictor responses to renal nerve stimulation, noradrenaline, phenylephrine and methoxamine were taken as the average of the values obtained during the ascending and descending frequencies and doses in the absence and presence of antagonist. The overall mean response was taken as the average value of renal vasoconstrictor responses to renal nerve stimulation and the agonists at all frequencies and doses respectively. All data was expressed as mean %change ± s.e.m. Statistical analysis of data was performed by a 2-way analysis of variance followed by Bonfferoni's post hoc test. The differences between the means were considered significant at the 5% level. Statistical analysis was performed using the statistical package, SUPERANOVA.

RESULTS

Baseline Values of Mean Arterial Pressure (MAP)

The baseline values of MAP in diabetic and normal SD rats were not significantly different ($P>0.05$). The average baseline values of MAP of AMP, 5-MeU, CEC and BMY7378 treated normal and diabetic rats were 135±3 and 130±4; 138±4 and 133±4; 136±4 and 135±3; and 130±2 and 125±5 mm Hg respectively. All animals showed a decrease in MAP values in the presence of low and high doses of antagonists but not at the significant level ($P>0.05$). The results are shown in Table 1.

Baseline Values of Renal Blood Flow (RBF)

The average base-line values of RBF in the cardiac failure induced SD rats treated with AMP, BMY7378, CEC and 5MeU were15.2±0.64, 14.2±0.54, 13.0±0.64 and 12.92±1.17 ml/min/kg respectively ($P>0.05$). Whereas, The average base-line values of RBF in the cardiac failure and diabetes induced SD rats treated with AMP, BMY7378, CEC and 5MeU were 14.5±1.2, 13.25±1.12, 14.21±0.08, and 13.05±1.5 ml/min/kg respectively ($P>0.05$). The results are shown in Table 2.

Renal Vasoconstrictor Responses

RNS produced frequency-dependent ($P<0.001$) decrease in renal blood flow in cardiac failure induced SD rats with or without diabetes. Similarly NA, PE and ME induced vasoconstrictor responses were dose dependent ($P<0.001$) in the both diabetic and non-diabetic rats with cardiac failure.

The low dose of AMP and BMY7378 did not show significant ($P>0.05$) change on the renal blood flow from the lowest to highest (1-10Hz) applied frequencies of RNS as compared to control in SD rats. Whereas the high doses of AMP and BMY7378 caused a significant ($P<0.05$) change on the renal blood flow from the lowest to highest (1-10Hz) applied frequencies of RNS as compared to control. However, high and low doses of 5MeU and CEC resulted in significant ($P<0.05$) change in the renal blood flow from the lowest to highest (1-10Hz) applied frequencies of RNS as compared to control in SD rats. Diabetic Sprague Dawley (SDD) group showed that high as well as low doses of all the antagonists i.e. AMP, 5MeU, CEC and BMY7378 caused a significant ($P<0.05$) change on the renal blood flow from the lowest to highest (1-10Hz) applied frequencies of RNS as compared to their respective controls. The results are shown in Figures 1, 2, 3 and 4.

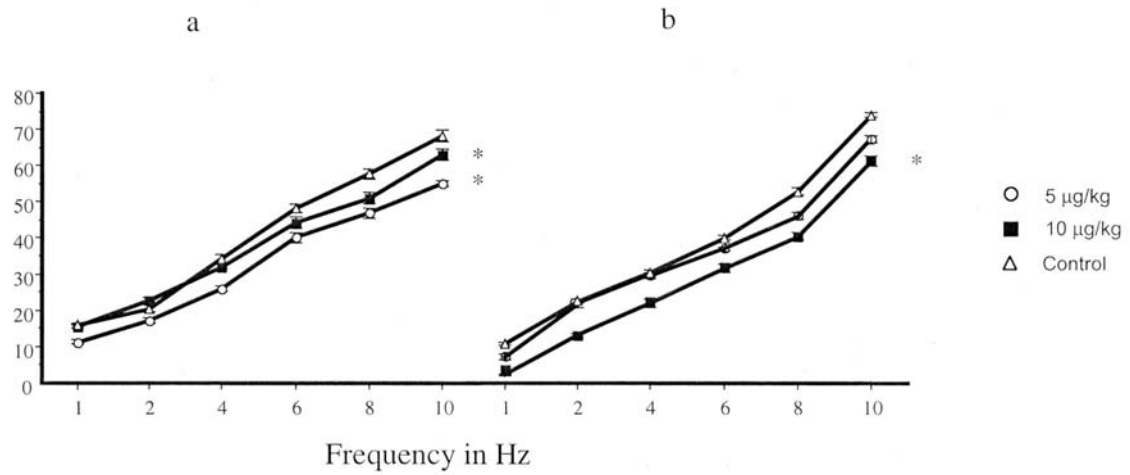

Fig. 1. Renal vasoconstrictor responses in cardiac failure SD (a) and SDD (b) rats to RNS in the absence and presence of 5 methylurapidil. The values are given as mean ± s.e.m., *indicates P<0.05 between control and treated groups.

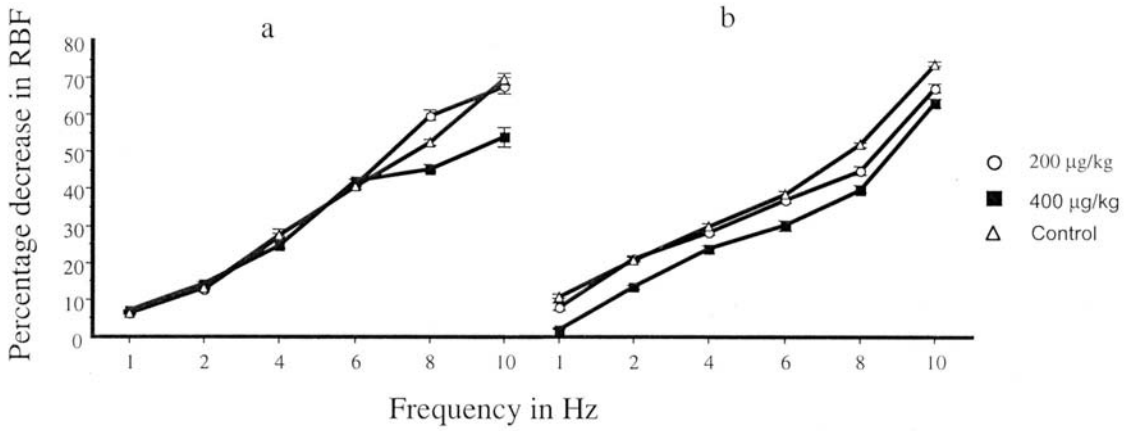

Fig. 2. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to RNS in the absence and presence of amlodipine. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

BMY7378 and AMP in both low and high doses caused a significant change (P<0.05) on the renal blood flow from the lowest to highest doses of NA as compared to control in SD rats. Whereas in case of 5MeU low dose does not cause any change in RBF from the lowest to highest doses of NA as compared to control in SD rats but high dose caused significant change (P<0.05) on the renal blood flow from the lowest to highest doses of NA as compared to control in SD rats. In CEC treated group of rats low dose caused significant change (P<0.05) on the renal blood flow from the lowest to highest doses of NA as compared to control in SD rats. Whereas high dose does not cause any change in RBF from the lowest to highest doses of NA as compared to control in SD rats. SDD group of animals showed that high as well as low doses of AMP, 5MeU, and BMY7378 caused a significant (P<0.05) change on the renal blood flow from the lowest to highest doses of NA as compared to their respective controls. Whereas CEC did not cause a significant (P>0.05) change on the renal blood flow from the lowest to ighest doses of as compared to their respective controls. The results are shown in Figures 5, 6, 7 and 8.

AMP, BMY7378, CEC and 5MeU in both low and high doses caused a significant change (P<0.05) in the renal blood flow from the lowest to highest doses of PE as compared to control in SD rats. SDD group of animals showed that high as well as low doses of AMP, 5MeU, CEC and BMY7378 caused a significant (P<0.05) change on the renal blood flow from the lowest to highest doses of PE as compared to their respective controls. The results are shown in Figures 9, 10, 11 and 12.

AMP, 5MeU and BMY7378, in both low and high doses caused a significant change (P<0.05) in the renal blood flow from the lowest to highest doses of ME as compared to control in SD rats. However, CEC in both low and high doses did not cause a significant change (P>0.05) in the renal blood flow from the lowest to highest doses of ME as compared to control in SD rats.

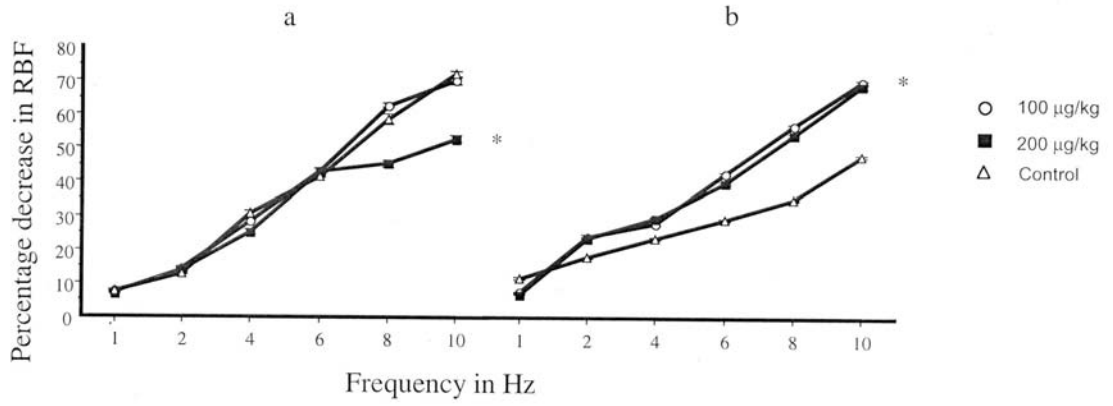

Fig. 3. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to RNS in the absence and presence of BMY7378. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups

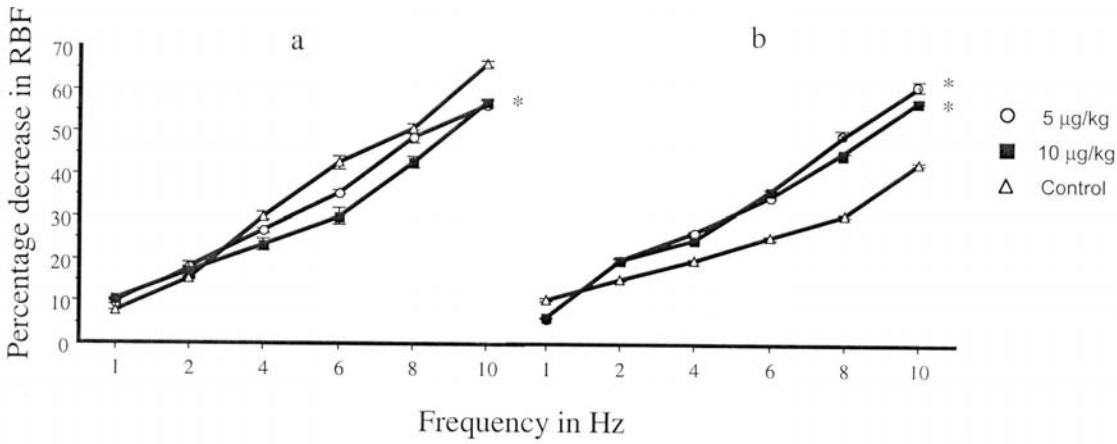

Fig. 4. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to RNS in the absence and presence of chloroethylclonidine. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

SDD group of animals showed that high as well as low doses of AMP, 5MeU, and BMY7378 caused a significant (P<0.05) change on the renal blood flow from the lowest to highest doses of ME as compared to their respective controls. However, high as well as low doses of CEC did not cause a significant (P>0.05) change on the renal blood flow from the lowest to highest doses of ME as compared to their respective controls. The results are shown in Figures 13, 14, 15 and 16.

DISCUSSION

Mean Arterial Pressure

Administration of AMP, 5MeU, CEC and BMY7378 caused slight reductions in MAP. However, these changes in MAP did not cause significant changes in RBF in all groups. The stability of RBF can be explained by the fact that the kidneys have an ability to auto regulate their own blood flow. Furthermore, direct RNS, exogenous NA, PE, and ME showed dose-related falls in RBF without any significant changes in MAP indicating their local effects within the kidney resistance vessels with little or no systemic effects (Sattar, 1993).

Renal vasoconstrictor Responses

It has been reported earlier that the renal artery constriction was regulated primarily by α_{1A}- adrenoceptor subtypes. This is based on its resistance to be alkylated by CEC (Piascik *et al*., 1994) and weakly antagonized by BMY7378 (Piascik *et al*., 1995). Sattar and Johns (1994a and b) reported that at the level of renal resistance vessels, the constriction was mediated by 5MeU sensitive α_1- adrenoceptors which are the α_{1A}-adrenoceptors and later Zhu *et al*. (1997) supported that α_{1A}- adrenoceptor is the major subtype in renal resistance arterioles.

In the heart failure, desensitization of β- adrenoceptors is related to a lower adrenergic responsiveness in heart. However, little is known about the α- adrenoceptors in the renal vasculature under this condition. Recent studies

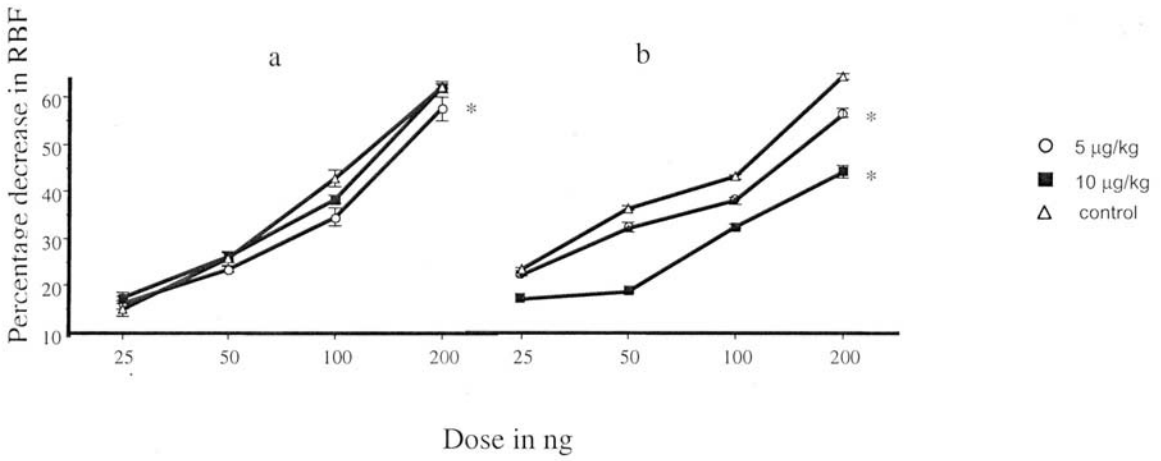

Fig. 5. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to NA in the absence and presence of 5 methylurapidil. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

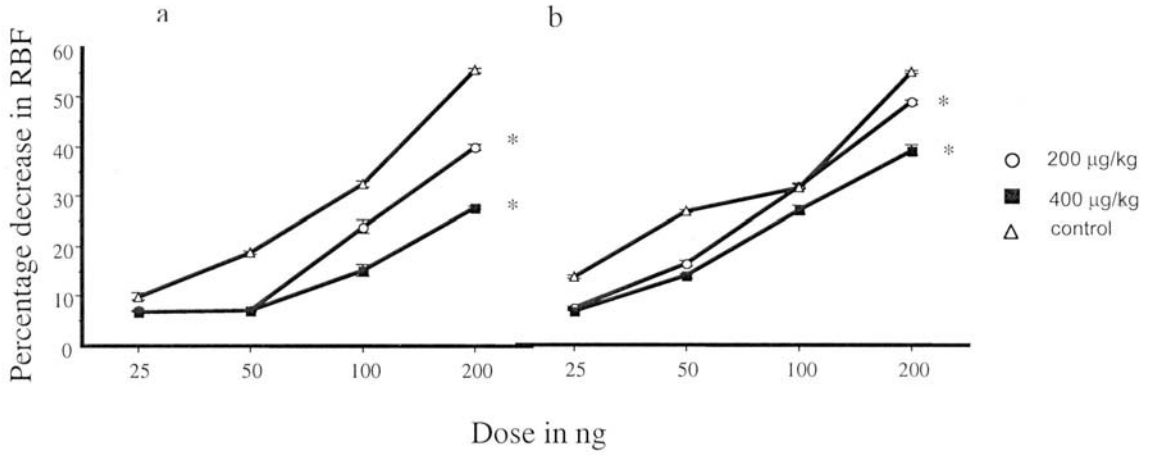

Fig. 6. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to NA in the absence and presence of amlodipine. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

have shown that α_{1D}- adrenoceptor function is maintained during congestive heart failure after myocardial infarction in rats and α_{1D}- adrenoceptors are the main receptors involved in aorta and carotid arteries, irrespective of congestive heart failure (Martinez and Dunbar, 1999). It has been indicated that the mRNAs for α_{1D}- adrenoceptor subtype exist in the renal artery (Piascik *et al*., 1994 and 1995; Guarino *et al*., 1996). This adrenoceptor subtype may play a significant role in the regulation of renal vasoconstriction (Hormetz *et al*., 1999). Besides playing a role in renal vasoconstriction, α_{1D}- adrenoceptors are also involved in the pathogenesis and maintenance of elevated blood pressure (Villalobos-Mollina *et al*., 1999).

The constrictor responses of vascular smooth muscles to vasoactive agents in chronic diabetic animals have been widely studied and it was shown that vascular responses to α- adrenoceptor agonists were unchanged, increased or decreased. The variability of the results of the vasoconstrictor responses could probably be due to the differences in procedures, strains, gender and age of rats used, and or duration of diabetes (Garcia *et al*., 1999). Diabetic neuropathy and nephropathy are the consequences of hyperglycemia (Raccah *et al*., 1996). The risk for the development of these alterations becomes higher with hypertension and heart failure (Schror, 1997). Furthermore, the ischemia induced vasospasm of the renal arterial blood vessels are mediated by α_1- adrenoceptors and are of importance for the loss of kidney functions (Eckert *et al*., 2000).

It is clear from the discussion that α_1- adrenoceptors play a major role in the regulation of the renal haemodynamics and hence, the present research focused on the α_1-

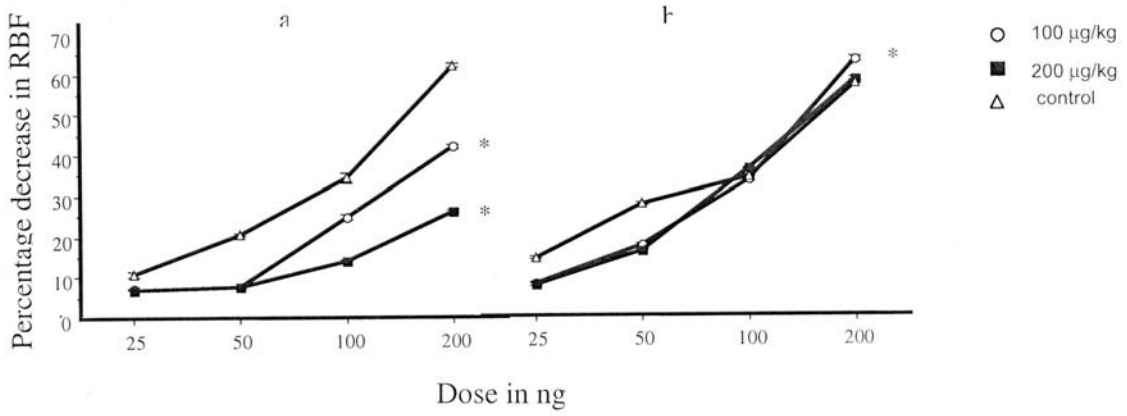

Fig. 7. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to NA in the absence and presence of BMY7378. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

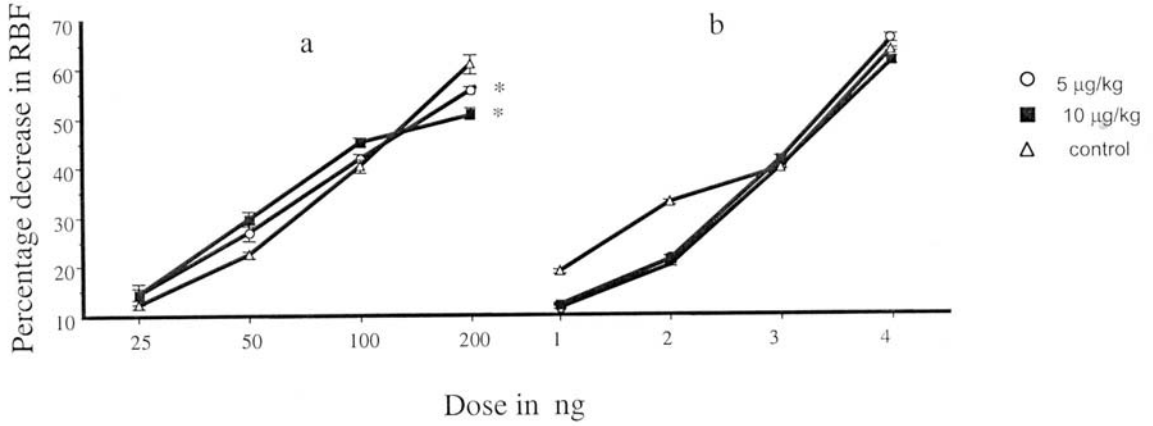

Fig. 8. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to NA in the absence and presence of chloroethylclonidine. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

adrenoceptor subtypes involved in the adrenergically induced vasoconstrictions in heart failure and its combination with diabetes in normotensive SD rats.

Electrical stimulations of the renal nerves at the increasing frequencies led to the graded reductions in renal blood flow. Previous investigators have also shown that in the normotensive rats, these responses are mediated by α_1-adrenoceptors. It is also demonstrated earlier that in 2K1C and DOCA- salt hypertensive group of rats, the calcium channel blockers attenuated the magnitude of the renal vasoconstrictor responses to nerve stimulation which became larger as the dose of AMP was raised (Sattar M.A and Johns E.J, 1994a). In the present study, in cardiac failure induced SD rats, the high dose of AMP, caused a significant blockage of vasoconstrictor responses induced by renal nerve stimulation. However, in the cardiac failure as well as diabetes induced SD rats, AMP caused a significant blockage of vasoconstrictor effects induced by renal nerve stimulation. These findings correlated well with the previous studies (Han *et al*., 1990, Sattar and Johns, 1996) that these α_1- adrenoceptors were dependent on the extra cellular calcium.

To further strengthen this view, 5 MeU a specific α_{1A}- adrenoceptor antagonist, was used to block the effect of renal nerve stimulation induced vasoconstriction. The results showed that in SD rats with and without diabetes the vasoconstrictor effects by RNS were suppressed in a dose related fashion as in case of AMP. These findings further suggested that α_{1A}-adrenoceptor subtype were involved in the neurally induced vasoconstriction.

In the presence of BMY7378 a α_{1D}- adrenoceptor antagonist, it was observed that in SD rats only the high dose blocked the vasoconstrictions. Similar results were obtained in cardiac failure and diabetes induced SD rats. These observations indicated that the renal

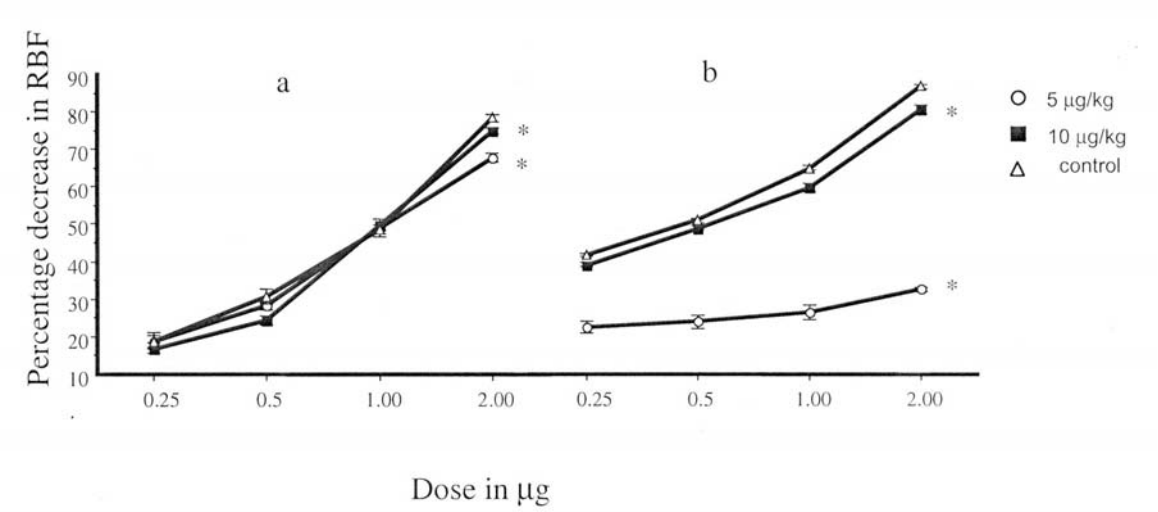

Fig. 9. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to PE in the absence and presence of 5 methylurapidil. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

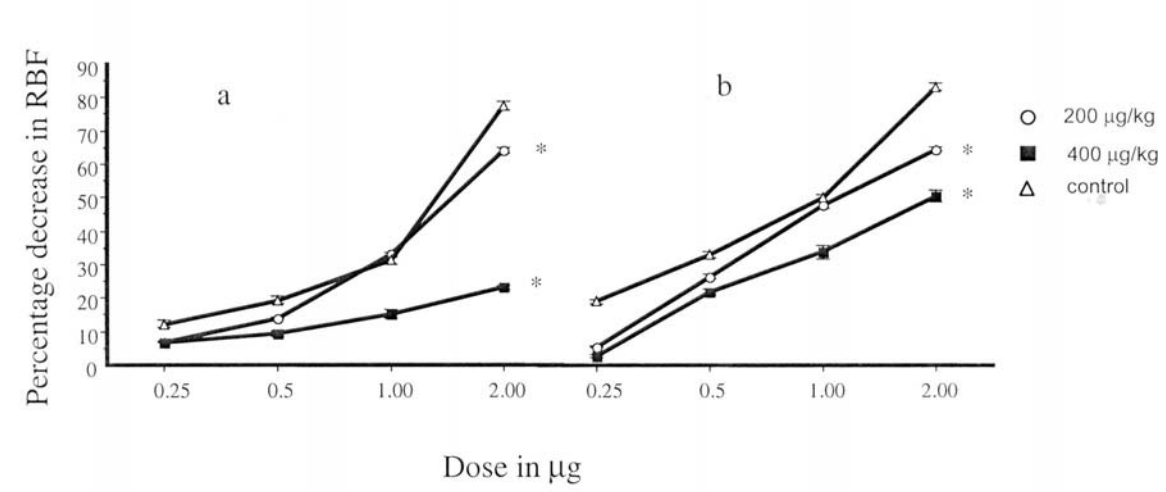

Fig. 10. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to PE in the absence and presence of amlodipine. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

vasoconstrictions in SD rats with cardiac failure and diabetes were not only mediated by α_{1A}- adrenoceptors as described by previous investigators (Blue *et al*., Sattar and Johns, 1994a and b) but also by α_{1D}- adrenoceptors based on their sensitivity to BMY 7378.

CEC an α_{1B}- adrenoceptor antagonist (Han *et al*., 1987, Piascik *et al*., 1995) that acts by alkylating α_{1B}- adrenoceptor and is an important tool in the sub classification of the α_1- adrenoceptors. In the present study high as well as low doses of CEC caused a significant reduction in the neurally induced vasoconstrictions in diabetic and non diabetic groups of SD rats with cardiac failure. This could have been attributed to the blockade of the unsaturated pre-synaptic α- adrenoceptors. This can be explained by the possibility of CEC, being and analogue of clonidine, to act pre-synaptically (Blue *et al*., 1992) and that these receptors could be of the α_{1B}- adrenoceptor subtype. There is a possibility that occupation of α_{1B}-adrenoceptors lead to an alteration in the properties of α_{1A}- adrenoceptors such that the normal agonist and antagonist interaction cannot occur. In this situation, the blockade of α_B-adrenoceptors would enhance the sensitivity of the remaining α_1- adrenoceptors such that a potentiation of the renal vasoconstrictor responses were seen. Another possibility is the activation of spare receptors which could have occurred with the blockade of α_{1B}- adrenoceptors. A similar situation was reported by Piascik *et al*. (1994) in which phenoxybenzamine incubated alone with the aortic rings completely abolish the response to phenylephrine, whereas following treatment with either SZL49, an α_{1A}-

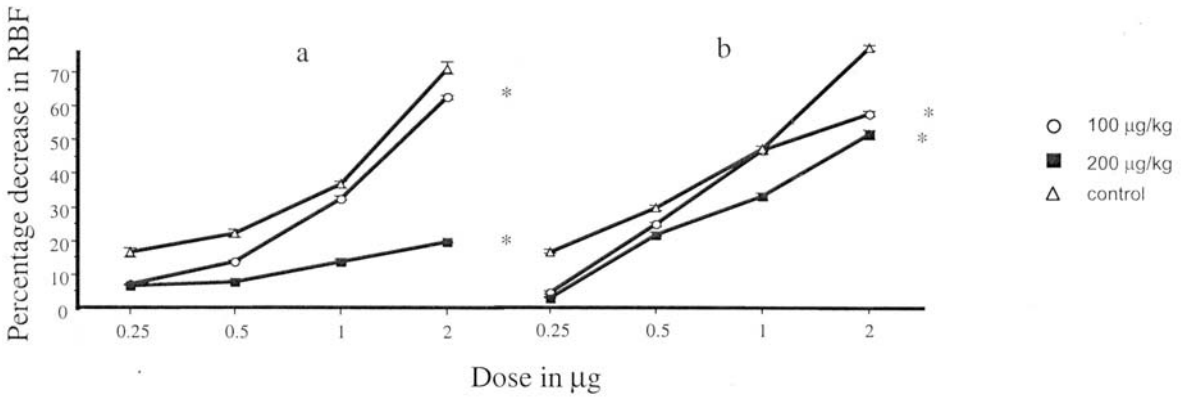

Fig. 11. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to PE in the absence and presence of BMY7378. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

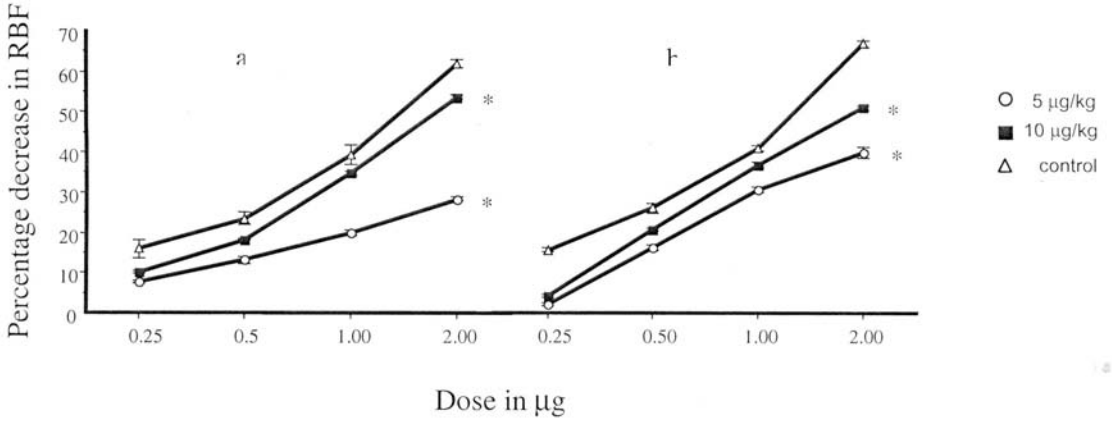

Fig. 12. Renal vasoconstrictor responses in cardiac failure induced SD rats to PE in the absence and presence of chloroethylclonidine. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

adrenoceptors antagonist, or CEC, a putative α_{1B}-adrenoceptor blocking agent, the actions of phenoxybenzamine, which can antagonize both the receptor subtypes was abolished.

Administration of noradrenaline caused dose dependent reductions in renal blood flow. The results showed that in cardiac failure induced SD as well as diabetic rats, AMP, BMY7378, and 5 MeU caused a significant attenuation of the vasoconstrictor effect, by noradrenaline where as CEC a specific α_{1B}-adrenoceptor antagonist in high and low dose did not exhibit any significant effects on the magnitude of noradrenaline induced vasoconstrictor responses.

According to Moriyama *et al*. (2000), the $\alpha_{1A/L}$-adrenoceptors primarily mediate the vasoconstrictor responses to noradrenaline, although other α_{1A}-adrenoceptor subtypes could also contribute in the mediation of the secondary contractile response to noradrenaline in the renal artery. RNase protection assay showed that the mean amount of α_{1A} mRNA was much greater than that of α_{1B} or α_{1D} mRNAs in both the main and branch renal arteries. Furthermore, *in situ* hybridization showed that all α_1- subtype mRNAs were localized in the smooth muscle cells of the tunica media of artery, and the distribution pattern of these three mRNAs in the main artery was the same as in the branch artery. However, the intensity of signals for α_{1D} and α_{1B} antisense RNAs probes was lower than that for the α_{1A} antisense RNA probe. Furthermore, CEC failed to inactivate the noradrenaline-induced contraction, and prazosin showed a relatively low affinity (Moriyama *et al*., 2000). These findings indicated that the α_{1B}-adrenoceptors are not involved in mediating renal vasoconstriction.

Administration of phenylephrine which activates both α_{1A} and α_{1B}- adrenoceptors, caused dose dependent reductions in renal blood flow in cardiac failure induced SD rats. The

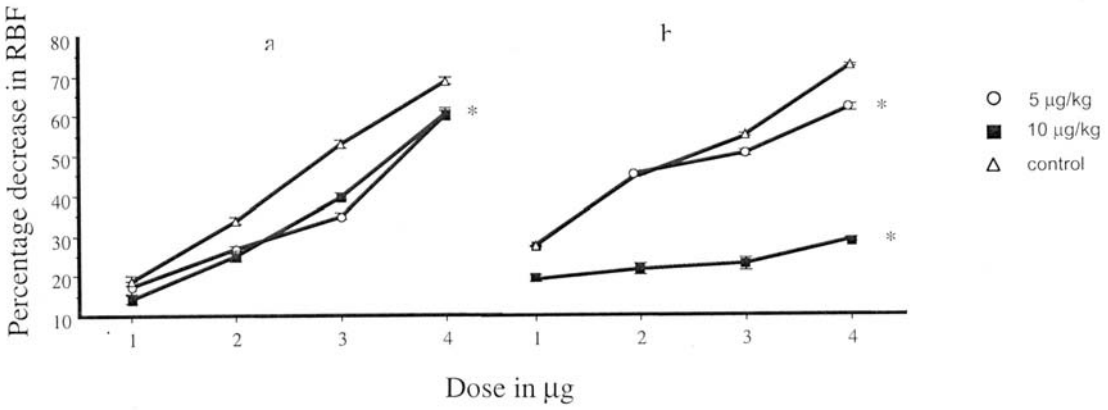

Fig. 13. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to ME in the absence and presence of 5 methylurapidil. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

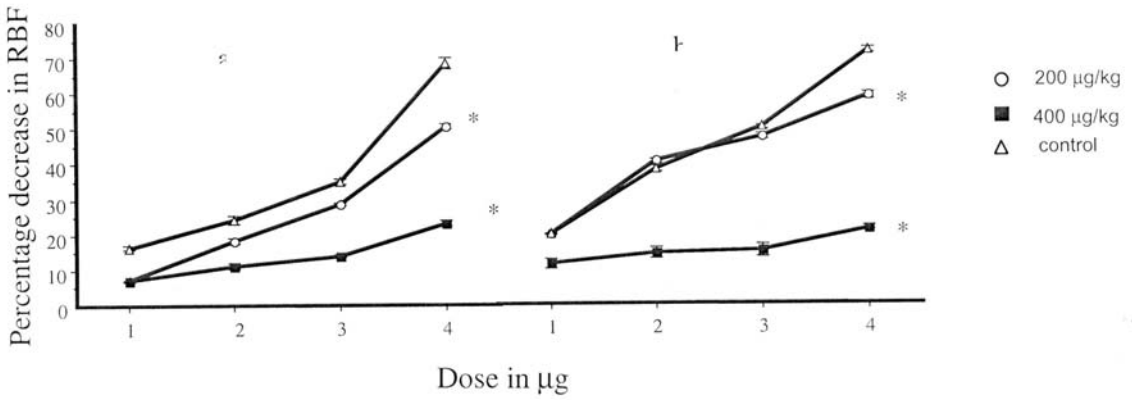

Fig. 14. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to ME in the absence and presence of amlodipine. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

renal vasoconstrictor responses to phenylephrine were significantly reduced by AMP, BMY7378 and 5MeU in cardiac failure induced rats but not by CEC. The results obtained are supported by the previous investigations (Sattar and Johns, 1996) and strengthen the view that the phenylephrine evoked renal vasoconstrictions were mediated by the subtype of α_1- adrenoceptors that were dependent on extracellular calcium influx, that is, the α_{1A}- adrenoceptors. To support this contention, studies using 5 MeU were utilized. 5MeU in the high and low doses significantly depressed the renal vasoconstrictor responses to exogenous phenylephrine. As phenylephrine is capable of exerting its action on α_1- adrenoceptors and an antagonism of the α_{1A}- adrenoceptors by 5MeU led to an attenuation of these vasoconstrictor responses, it could be suggested that these responses are mediated by the α_{1A}- adrenoceptors. On the other hand, CEC did not exhibit any significant effects on the magnitude of the phenylephrine induced renal vasoconstrictor responses. This it can be argued, that the failure of CEC to manifest any meaningful changes would suggest a minor role of the α_{1B}- adrenoceptors in mediating the phenylephrine induced vasoconstrictor responses. These studies combined provides further support that in cardiac failure SD rats, α_{1A}- adrenoceptors are functionally important. In cardiac failure and diabetes induced SD rats, CEC also reduced the vasoconstrictor effect induced by phenylephrine. Similar findings were obtained when AMP, BMY7378 and 5MeU was utilized. The close renal arterial administration of methoxamine, a putative α_{1A}- adrenoceptor antagonist (Tsujimoto *et al.*, 1989) similarly resulted in dose dependent reductions in renal blood flow in cardiac failure induced SD rats. AMP, BMY7378 and 5 MeU (high doses) administration resulted in suppression of methoxamine induced renal vasoconstrictions in all groups. However, administration of CEC did not show a significant reduction in methoxamine induced renal vasoconstriction in cardiac failure SD rats. This observation further strengthen the view that α_{1A}- adrenoceptors are involved in renal

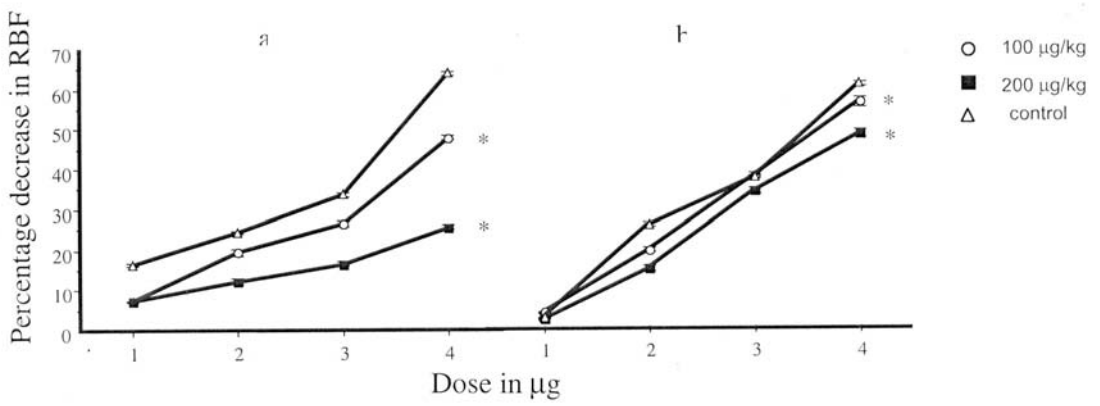

Fig. 15. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to ME in the absence and presence of BMY7378. The values are given as mean ± s.e.m., * indicates P<0.05 between control and treated groups.

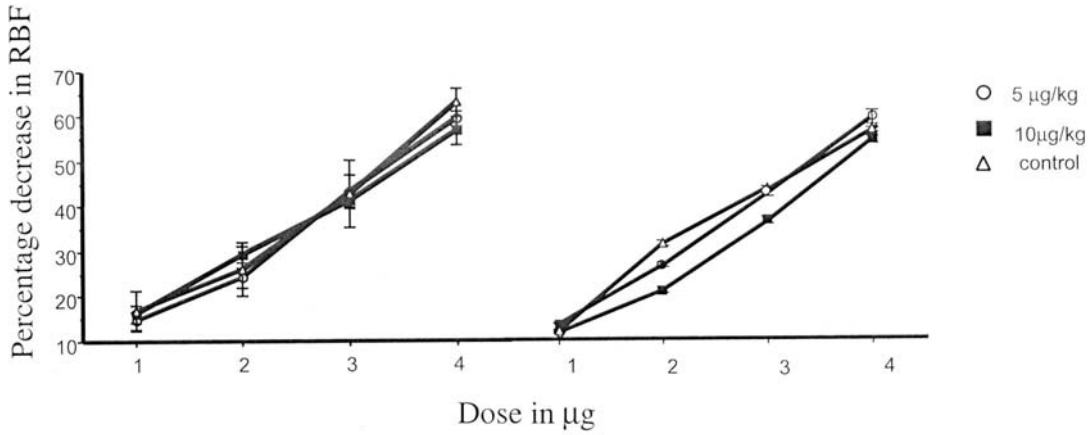

Fig. 16. Renal vasoconstrictor responses in cardiac failure induced SD (a) and SDD (b) rats to ME in the absence and presence of chloroethylclonidine. The values are given as mean ± s.e.m.

vasculature of cardiac failure and diabetes induced SD rats. However, interestingly in all groups of animals CEC, resulted in minor statistically non-significant reductions in vasoconstrictor responses induced by methoxamine. One possible explanation for this observation is that there may be a complex interaction between the subtypes of adrenoceptors in cardiac failure as well as diabetes induced SD rats.

These findings supported that differences in α_1-adrenoceptor populations and distribution in blood vessels are dependent on the pathological state (LeTran and Forster, 1997). The finding from this study suggest that the α_{1A} and α_{1D}- adrenoceptor mediate the adrenergically induced renal vasoconstrictor responses in cardiac failure SD rats with and without diabetes.

CONCLUSIONS

These studies collectively provide further support that in cardiac failure SD with and without diabetes α_{1A}-adrenoceptors are functionally important. The pathological conditions i.e., diabetes and cardiac failure can cause the down regulation of α_{1A}- adrenoceptors. The renal vasoconstrictions in SD rats with cardiac failure and diabetes were not only mediated by α_{1A}- adrenoceptors but also by α_{1D}-adrenoceptors based on their sensitivity to BMY 7378.

α_{1B}-adrenoceptors are not involved in mediating renal vasoconstrictions in these animal models. In SD cardiac failure rats, diabetes did not influence the functionality of the α_1-adrenoceptor subtypes in the regulation of renal haemodynamics.

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