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The contribution of adrenoceptor subtype(s) in the renal vasculature of diabetic spontaneously hypertensive rats

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1 Diabetes and hypertension are both associated with an increased risk of renal disease and are associated with neuropathies, which can cause defective autonomic control of major organs including the kidney. This study aimed to examine the α_1-adrenoceptor subtype(s) involved in mediating adrenergically induced renal vasoconstriction in a rat model of diabetes and hypertension.

2 Male spontaneously hypertensive rats (SHR), 220–280 g, were anaesthetized with sodium pentobarbitone 7-day poststreptozotocin (55 mg kg^{-1} i.p.) treatment. The reductions in renal blood flow (RBF) induced by increasing frequencies of electrical renal nerve stimulation (RNS), close intrarenal bolus doses of noradrenaline (NA), phenylephrine (PE) or methoxamine were determined before and after administration of nitrendipine (Nit), 5-methylurapidil (5-MeU), chloroethylclonidine (CEC) and BMY 7378.

3 In the nondiabetic SHR group, mean arterial pressure (MAP) was 146 ± 6 mmHg, RBF was 28.0 ± 1.4 ml min^{-1} kg^{-1} and blood glucose was 112.3 ± 4.7 mg dl^{-1}, and in the diabetic SHR Group, MAP was 144 ± 3 mmHg, RBF 26.9 ± 1.3 ml^{-1} min kg^{-1} and blood glucose 316.2 ± 10.5 mg dl^{-1}. Nit, 5-MeU and BMY 7378 blunted all the adrenergically induced renal vasoconstrictor responses in SHR and diabetic SHR by 25–35% (all $P<0.05$), but in diabetic rats the responses induced by RNS and NA treated with 5-MeU were not changed. By contrast, during the administration of CEC, vasoconstrictor responses to all agonists were enhanced by 20–25% (all $P<0.05$) in both the SHR and diabetic SHR.

4 These findings suggest that α_{1A} and α_{1D}-adrenoceptor subtypes contribute in mediating the adrenergically induced constriction of the renal vasculature in both the SHR and diabetic SHR. There was also an indication of a greater contribution of presynaptic adrenoceptors, that is, α_{1B}-, and/or α_2-subtypes.
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Keywords: Spontaneously hypertensive rats; streptozotocin induced diabetes; renal vasoconstriction; alpha-adrenoceptor subtypes

Abbreviations: CEC, chloroethylclonidine; Dia-SHR, diabetic spontaneously hypertensive rat; MAP, mean arterial pressure; ME, methoxamine; 5-MeU, 5-methylurapidil; NA, noradrenaline; Nit, nitrendipine; PE, phenylephrine; RBF, renal blood flow; RNS, renal nerve stimulation; SHR, spontaneously hypertensive rat

Introduction

The renal sympathetic nerves are increasingly considered as being important in regulating renal hemodynamic and tubular function to maintain body fluid homeostasis and thus blood pressure (DiBona & Kopp, 1997). The adrenoceptors that mediate the action of NA released from the varicosities along the sympathetic nerves exist in several subtypes, but at the renal vasculature and epithelial cells of the nephron it is the α_1-adrenoceptor subtype that is functionally relevant (Johns & Manitius, 1986). It has been reported by Feng *et al.* (1991) that the density of α_1-adrenoceptors is highest in the cortex and decreases from cortex to papilla. Molecular biological studies and cloning approaches have clearly defined at least three subtypes of α_1-adrenoceptor, namely α_{1A}, α_{1B} and α_{1D} (Hieble *et al.*, 1995) and even more may exist and have some functional role. Multiple adrenoceptor subtypes have been shown to be present in the kidney (Minneman *et al.*, 1988; Han *et al.*, 1990; Feng *et al.*, 1991; Jackson *et al.*, 1992). α_{1A}- and α_{1B}-adrenoceptors exist to an almost equal extent in the cortex and outer stripe of the outer medulla, but with the α_{1B}-subtype predominating in the inner stripe of the outer medulla, while at the proximal tubules they are expressed at approximately equal levels (Feng *et al.*, 1991).

Within the kidney, α_{1A}-adrenoceptors have been consistently found to predominate over the α_{1B} and α_{1D}, both in terms of density and functionality (Clarke *et al.*, 1990; Han *et al.*, 1990; Eltze *et al.*, 1991; Blue *et al.*, 1992; 1995; Elhawary *et al.*, 1992; Stassen *et al.*, 1998). Activation of α_{1A}-adrenoceptors causes constriction of the renal vascular resistance bed (afferent and efferent arterioles) in the Wistar, Stroke-Prone Spontaneously Hypertensive Rats (SHRSP), Two kidney One Clip (2K1C) and Deoxycorticosterone acetate-Salt (DOCA-Salt) hypertensive rats, while the α_{1B} adrenoceptor subtype seems to play a lesser role (Sattar & Johns, 1994a, b). Other studies reported

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that the renal adrenergic vasoconstrictor responses are mediated by α_{1A}- (Elhawary *et al*., 1992; Blue *et al*., 1995; Villalobos-Molina & Ibarra, 1997) and/or *via* α_{1D}-adrenoceptor subtypes (Villalobos-Molina & Ibarra, 1996; 1997), although Piascik *et al*. (1995) suggested that the α_{1D}-adrenoceptor subtype did not appear to play a role in mediating contraction of this vessel.

Hypertension and diabetes are conditions that often occur together and it is the vascular complications of this state, which are the major cause of morbidity and mortality. In hypertension, alterations in blood vessel and kidney structure and function occur and there is an enhancement of vascular and renal α-adrenoceptor function (Takata & Kato, 1996). Type I diabetes is associated with autonomic neuropathy which may lead to deficiencies in the neural regulation of major vascular beds, including the kidney. Diabetes is also identified as a risk factor for the development and progression of cardiovascular disease, and thus one of the potential risk factors to be assessed in this study is renal vascular disease. During the development of diabetes there have been reports of an enhancement (Jackson & Carrier, 1981), no change (Kam *et al*., 1996) or inhibition (Beenen *et al*., 1996) of adrenergically mediated vasoconstrictor responses. Frequently diabetes is associated with the development of hypertension, but to date there have been no detailed studies concerned with the distribution and/or functionality of α_1-adrenoceptor subtypes in such a combined diabetes–hypertension state. This study aimed to determine whether the heterogeneous population of α_1-adrenoceptor subtypes, which contribute to the renal adrenergic vasoconstrictor responses under normal conditions, are altered in any way in a model of combined diabetes and hypertension. To this end, we utilized the streptozotocin diabetic spontaneously hypertensive rat (SHR) and examined the vascular responses to a range of endogenous and exogenous vasoconstrictor agents.

Methods

Diabetic model

Male SHR 250–300 g of body weight were injected with 55 mg kg^{-1} streptozotocin (Sigma, U.S.A.) dissolved in saline intraperitoneally and housed in groups of four for 3 days (Patel & Zhang, 1994). For the first 48 h after injection, the rats received 5% of glucose in the drinking water and thereafter water *ad libitum* and regular chow. On the third day, the rats were housed individually in metabolic cages, and daily water intake and urine output were measured from days 4 to 7, while body weights were measured every other day until day 7 when the acute study was undertaken. Fasting blood glucose was estimated on the day of acute experiment using a Peridochrom Glucose kit (Boehringer Mannheim). Only rats with blood glucose of 200 mg dl^{-1} or above were included in this study.

General preparation Rats were anaesthetized with sodium pentobarbitone 60 mg kg^{-1} intraperitoneally and supplemented with 12.5 mg kg^{-1} h^{-1} intra-arterially throughout the experiment, given in a continuous infusion of saline (150 mmol NaCl) at 6 ml h^{-1}. After tracheotomy, the jugular vein was cannulated for saline infusion and bolus doses of anaesthetic as required and the carotid artery was cannulated for blood pressure measurement (Statham P23 ID pressure transducer attached to a Grass Model 79E polygraph). The kidney was exposed *via* a midline abdominal incision, the renal artery was cleared and a bipolar electromagnetic flow probe (Carolina EP100 series connected to a Carolina flowmeter and polygraph) was fitted to allow direct recording of renal blood flow (RBF). The renal nerves were identified, sectioned and bipolar electrodes were connected to permit direct renal nerve stimulation (RNS). The iliac artery was cleared and a cannula was inserted such that its tip lay in the aorta just rostral to the left renal artery, which allowed close intrarenal arterial administration of saline and drugs. A 0.4 ml blood sample was withdrawn *via* the carotid artery for blood glucose measurement, centrifuged for 2 min at 6000 rpm, the plasma was taken and the blood cells were resuspended in an equal volume of saline and re-infused into the animal. Upon completion of the surgery, 2 ml of saline was given i.v. as a primer and the animals were allowed to stabilize for 1 h before the commencement of experiments (Sattar & Johns, 1991).

Experimental protocols Eight groups of rats (each of 5–8 animals) were used, groups 1–4 were SHR and group 5–8 were diabetic spontaneously hypertensive rats (Dia-SHR).

The experiment was divided into three parts. In Part I, the animals were infused with vehicle as a control, while in Parts 2 and 3 they were infused i.v. with low and high doses of drug, respectively. A sequence of frequency–response curves to RNS, and dose–response curves to NA, PE and methoxamine (ME) were undertaken in each part of the experiment. RNS and administration of agonists was carried out twice in an ascending followed by descending order of frequencies or doses. The reduction in RBF to direct electrical RNS at 1, 2, 4, 6, 8 and 10 Hz at 15 mV and 2 ms, bolus doses of NA, 25, 50, 100 and 200 ng kg^{-1}, PE, 0.25, 0.50, 1 and 2 μg kg^{-1}, and ME 1, 2, 3 and 4 μg kg^{-1} were determined before and 15 min after administration of bolus doses of vehicle and antagonist. The antagonists were given as an initial bolus dose followed by a continuous infusion of one 40th of that dose per hour until completion of the agonist/stimulation protocol and thereafter the second dose of compound was used. Nitrendipine (Nit) was given as a bolus dose of 200 and 400 μg kg^{-1} followed by an infusion of 5 and 10 μg kg^{-1} h^{-1}, respectively (Groups 1 and 5), while 5-methylurapidil (5-MeU, Groups 2 and 6), chloroethylclonidine (CEC, Groups 3 and 7), were given as bolus doses of either 5 or 10 μg kg^{-1} followed by a continuous infusion of 0.125 or 0.25 μg kg^{-1} h^{-1}, respectively, and BMY 7378 was administered as a bolus dose of 100 or 200 μg kg^{-1} plus a continuous infusion of 2.5 and 5 μg kg^{-1} h^{-1}, respectively (Groups 4 and 8), respectively.

Drugs Drugs used in this study were prepared as stock solutions as follows:

Streptozotocin (Sigma) was dissolved in saline and used immediately. 5-MeU (Research Biochemicals International, U.S.A.) was made up in 0.04 M lactic acid at 100 μg ml^{-1}; CEC (Research Biochemicals International, U.S.A.) was made up in saline 100 μg ml^{-1}, Nit (Research Biochemicals International, U.S.A.) was made up in a mixture of PEG-400 : glycerol : distilled water = 969 : 60 : 100 at 1000 μg ml^{-1} and BMY 7378 (Research Biochemicals International, U.S.A.) was made in saline at 1000 μg ml^{-1}. All solutions were refrigerated and used

within 3 days. These stock solutions were diluted in saline before being used. NA (Levophed, Sanofi Winthrop, U.K.), PE (BASF Pharm, Knoll, U.K.) and ME (Vasoxine, Calmic Medical Division, U.K.) were diluted freshly from the ampules containing 10, 10 and 20 $\mu g\,ml^{-1}$ respectively on the day of experiment.

Statistics The renal vasoconstrictor responses to RNS, NA, PE and ME were taken as an average of the two decreases in RBF obtained at each frequency or agonist dose during the sequence of ascending and descending series of stimulations or drug administrations. (Sattar & Johns, 1994a). The mean renal vasoconstrictor responses for each sequence of experiments in the presence of vehicle was taken as 100% and the percentage changes in the presence of each dose of antagonist were calculated. Data, mean ± s.e.m., were analyzed by three-way ANOVA (Superanova, Abacus, CA, U.S.A.) followed by Duncan *post hoc* test and multirange *T*-test, and significance was taken at the 5% level.

Results

General observations

The diabetic state was confirmed by the presence of polyuria and polydipsia in the rats within the week after injection of streptozotocin. Serum glucose of the diabetic SHR was $316.2 \pm 10.5\,mg\,dl^{-1}$ and for the SHR it was $112.3 \pm 4.7\,mg\,dl^{-1}$.

Basal blood pressure and RBF for the different groups of rats are given in Table 1. The BMY treated group of rats had a significantly higher baseline MAP in diabetic compared to the nondiabetic group of rats ($P<0.05$), while other groups were not different. RBF was similar in all groups of animals.

Renal vasoconstrictor responses

The magnitudes of the reductions in RBF induced by RNS, intrarenal arterial NA and PE infusions (Table 2) were all larger in the diabetic compared to the nondiabetic SHR (all $P<0.05$). By contrast, ME given intrarenal arterially caused similar decreases in RBF in both group of SHR (Table 2).

Effect of Nit

In the presence of both the low and high doses of Nit, the renal vasoconstrictor responses to RNS were blunted and the dose–reponse relationships were shifted to the right (all $P<0.001$) in the diabetic and nondiabetic SHR (Figure 1). The NA induced decreases in RBF were all significantly ($P<0.001$) blunted by the two doses of Nit in both the diabetic and nondiabetic groups of SHR (Figure 1). There were no differences in the degree of attenuation caused by the two doses of Nit in either group of SHR. Both doses of Nit blunted the renal vasoconstrictor responses to PE and ME ($P<0.001$) to a similar degree in the diabetic and nondiabetic SHR (Figure 1).

Effect of 5-MeU

The reductions in RBF caused by the RNS and NA infusions were unchanged by the presence of both doses of 5-MeU in the diabetic SHR, but caused dose-related decreases in the magnitude of the responses in the nondiabetic SHR ($P<0.001$, Figure 2). Interestingly, the 5-MeU caused a dose-related inhibition of both PE and ME mediated responses (Figure 2) in the diabetic as well as the nondiabetic SHR groups ($P<0.001$).

Effect of CEC

Figure 3 shows that administration of CEC caused dose-dependent increases (all $P<0.05$) in the magnitudes of the renal vasoconstrictor responses obtained by stimulation of the renal nerves, and intrarenal arterial infusions of NA, PE and ME in the diabetic SHR. However, in the nondiabetic SHR, the nerve and agonist induced reductions in RBF were unaffected by either dose of CEC.

Table 1 Baseline values of RBF and MAP in the nitrendipine, 5-methylurapidil, chloroethylclonidine and BMY 7378 treated SHR and Dia-SHR (*$P<0.05$, $n=5$–8)

Parameters	*Rat conditions*	*Nitrendipine treated*	*5-MeU treated*	*CEC treated*	*BMY 7378 treated*
MAP (mmHg)	Normal	141 ± 9	146 ± 6	145 ± 4	184 ± 6*
	Diabetic	136 ± 4	135 ± 5	144 ± 4	150 ± 4
RBF ($ml\,min^{-1}\,kg^{-1}$)	Normal	27.4 ± 1.2	28.0 ± 1.4	25.5 ± 1.1	31.7 ± 1.3
	Diabetic	23.8 ± 1.1	24.4 ± 2.1	26.9 ± 1.3	32.7 ± 1.9

Table 2 The mean percentage decreases in RBF induced adrenergically in the BMY 7378, CEC, 5-MeU and nitrendipine treated SHR rats (*$P<0.05$, $n=5$–8)

Inducer	*BMY-7378*		*CEC*		*5-MeU*		*Nitrendipine*	
	Nondiabetic	*Diabetic*	*Nondiabetic*	*Diabetic*	*Nondiabetic*	*Diabetic*	*Nondiabetic*	*Diabetic*
RNS	13.6 ± 1.0	22.3 ± 1.6*	30.2 ± 1.9	33.9 ± 1.9*	18.1 ± 1.3	30.2 ± 1.7*	19.5 ± 1.6	20.7 ± 1.8
NA	23.5 ± 1.8	30.2 ± 2.1*	32.1 ± 3.0	41.3 ± 2.4*	28.9 ± 2.2	42.6 ± 2.4*	15.6 ± 1.5	26.2 ± 2.6*
PE	24.2 ± 2.3	28.2 ± 2.5*	35.0 ± 3.3	41.5 ± 2.9*	23.4 ± 2.5	33.2 ± .9*	19.7 ± 2.0	34.1 ± 2.8*
ME	35.4 ± 1.9	40.0 ± 2.2	59.9 ± 4.0	57.1 ± 3.0	24.6 ± 2.6	34.3 ± 2.7*	18.9 ± 1.9	35.9 ± 2.7

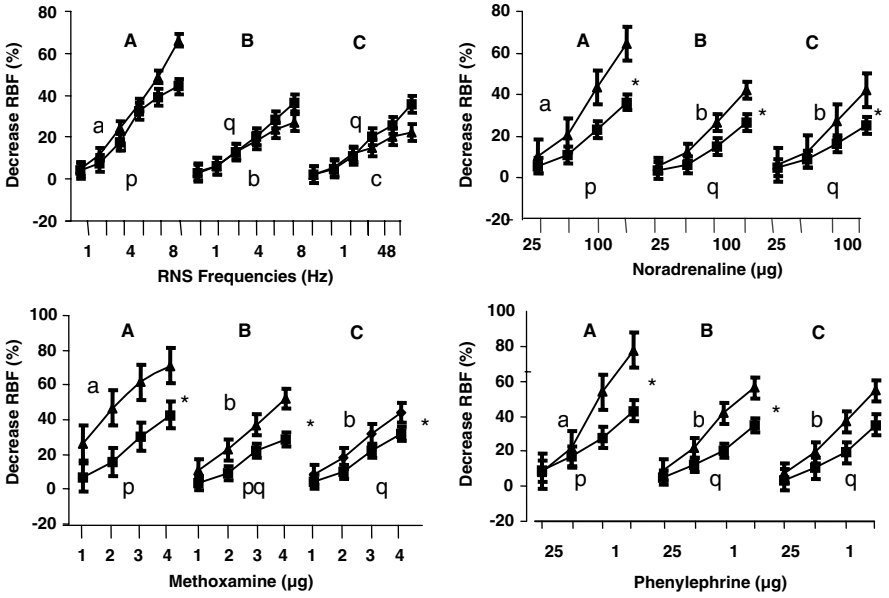

Figure 1 Renal vasoconstrictor responses to RNS, NA, ME and PE in the absence (a) and presence of low (b) and high (c) doses of Nit in diabetic (filled triangles) and nondiabetic (filled squares) SHRs. The values are given as means ± s.e.m. *Indicates $P<0.05$ between nondiabetic and diabetic rats; a and b, and p and q indicate $P<0.05$ between doses of antagonist in nondiabetic and diabetic rats respectively.

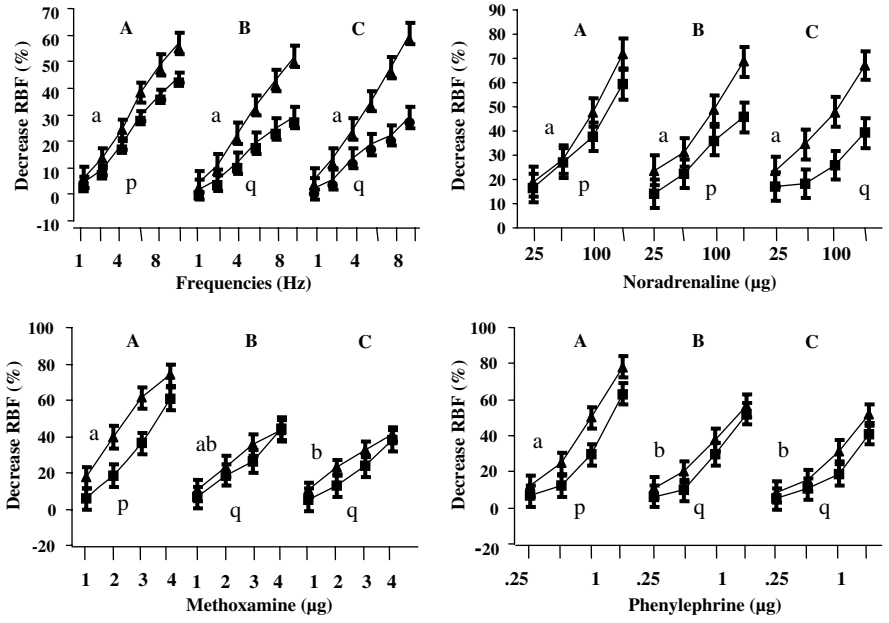

Figure 2 Renal vasoconstrictor responses to RNS, NA, ME and PE in the absence (a) and presence of low (b) and high (c) doses of 5-MeU in diabetic (filled triangles) and nondiabetic (filled squares) SHRs. The values are given as means ± s.e.m. *Indicates $P<0.05$ between nondiabetic and diabetic rats; a and b, and p and q indicate $P<0.05$ between doses of antagonist in nondiabetic and diabetic rats respectively.

Effect of BMY 7378

BMY 7378 enhanced ($P<0.05$) the renal nerve-induced vasoconstrictor responses in the diabetic SHR at both the low and high doses of the antagonist (Figure 4). By contrast, in the nondiabetic SHR, BMY 7378 decreased ($P<0.05$) the nerve-mediated vasoconstrictions. In terms of the NA-induced reductions in RBF, the higher dose of BMY 7378 significantly ($P<0.05$) blunted the responses in both the diabetic and nondiabetic SHR ($P<0.05$, Figure 4). BMY 7378 significantly ($P<0.001$) decreased the renal vasoconstrictor responses following the ME administration to a similar degree with both doses of BMY 7378 and to the same extent in the diabetic and nondiabetic SHR. A similar pattern was obtained in the PE dose–response relationships, that is, the renal vasoconstrictor responses were blunted in both the diabetic and nondiabetic SHR by both the low and high doses of BMY 7378.

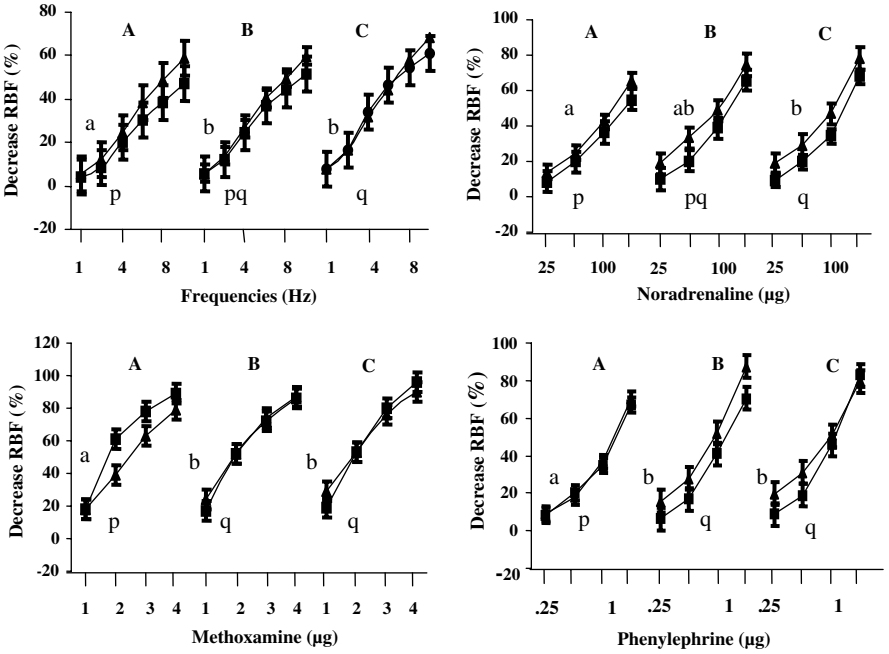

Figure 3 Renal vasoconstrictor responses to RNS, NA, ME and PE in the absence (a) and presence of low (b) and high (c) doses of CEC in diabetic (filled triangles) and nondiabetic (filled squares) SHRs. The values are given as means ± s.e.m. *Indicates $P<0.05$ between nondiabetic and diabetic rats; a and b, and p and q indicate $P<0.05$ between doses of antagonist in nondiabetic and diabetic rats respectively.

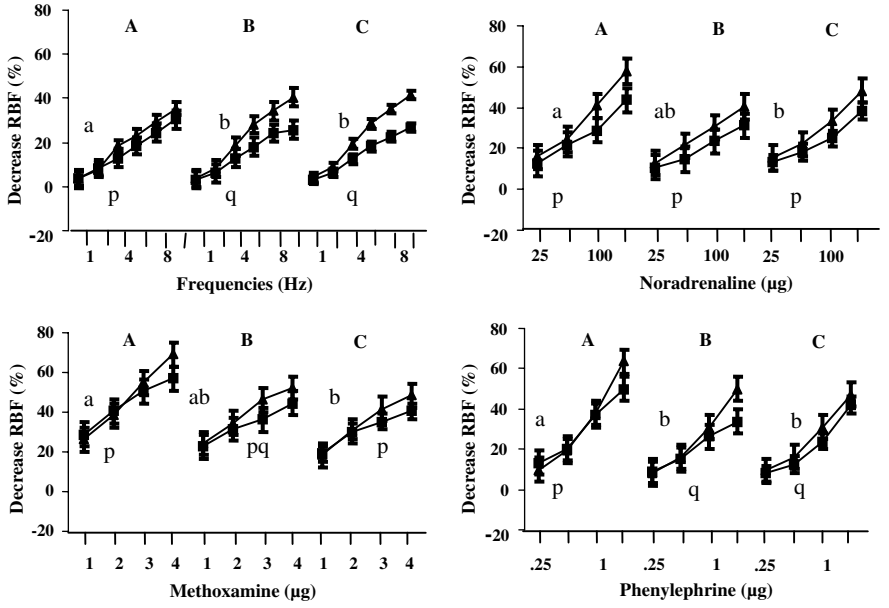

Figure 4 Renal vasoconstrictor responses to RNS, NA, ME and PE in the absence (a) and presence of low (b) and high (c) doses of BMY 7378 in diabetic (filled triangles) and nondiabetic (filled squares) SHRs. The values are given as means ± s.e.m. *Indicates $P<0.05$ between nondiabetic and diabetic rats; a and b, and p and q indicate $P<0.05$ between doses of antagonist in nondiabetic and diabetic rats respectively.

In summary, the adrenergically induced renal vasoconstrictor responses in diabetic SHR were greater when compared to nondiabetic rats. These responses in nondiabetic SHRs were attenuated by Nit, 5-MeU and by BMY 7378. In the presence of CEC, the NA, PE and ME induced responses were unchanged while RNS induced responses were enhanced.

In the diabetic SHR, all the renal vasoconstrictor responses were attenuated by Nit but 5-MeU attenuated the responses induced by PE and ME, while not affecting the RNS and NA responses. BMY 7378 blunted the exogenous agonist while enhancing RNS induced vasoconstrictions. However, all vasoconstrictor responses were enhanced in the presence of chloroethylclonidine (Table 3).

Discussion

This study set out to examine the contribution of the α_1-adrenoceptor subtype (s) in controlling the adrenergically

Table 3 Summary of renal vasoconstrictor responses induced by RNS, NA, PE and ME in the presence of BMY 7378, 5-MeU, CEC and Nitrendipine (Nit) in diabetic and nondiabetic SHRs

	Nondiabetic				*Diabetic*			
Inducers	*BMY 7378*	*CEC*	*5-MeU*	*Nit*	*BMY 7378*	*CEC*	*5-MeU*	*Nit*
RNS	↓	↑	↓	↓	↑	↑	↑↓	↓
NA	↓	↑↓	↓	↓	↓	↑	↑↓	↓
PE	↓	↑↓	↓	↓	↓	↑	↓	↓
ME	↓	↑↓	↓	↓	↓	↑	↓	↓

induced renal vasoconstrictor responses in anaesthetised rats, which were both diabetic and hypertensive. Renal vasoconstrictor responses were induced by a variety of means. Firstly, direct RNS in which both pre- and postsynaptic α-adrenoceptors would be involved in determining the level the neurotransmission process (Schwartz & Malik, 1989). Secondly, the administration of exogenous NA, a mixed agonist, thirdly, PE, a synthetic nonselective α_{1A}-, α_{1B}-, α_{1D}-adrenoceptor agonist (Summers, 1984; Boer *et al*., 1989) and fourthly, ME, a relatively selective α_{1A}-adrenoceptor agonist (Tsujimoto *et al*., 1989).

The proposed mechanism whereby CEC exerts its effect is to alkylate and inactivate the α_{1B}-adrenoceptor subtype (Morrow & Creese, 1986; Johnson & Minneman, 1987; Han *et al*., 1987; Minneman *et al*., 1988; Piascik *et al*., 1992). The sensitivity of α_1-adrenoceptors to CEC has been reported to be $\alpha_{1B} > \alpha_{1D} \gg \alpha_{1A}$ (Perez *et al*., 1994). On the other hand, 5-MeU has been found to selectively inhibit α_{1A}-adrenoceptors (Gross *et al*., 1988; Minneman *et al*., 1988; Michel *et al*., 1989; Schwinn & Lomasney, 1992; Faure *et al*., 1994; Forray *et al*., 1994). Nit is an L-type Ca^{2+} channel blocker and these channels appear to be coupled to the α_{1A}-adrenoceptor and are opened when the receptor is activated (Han *et al*., 1987; Suzuki *et al*., 1990). BMY 7378 (Goetz *et al*., 1995; Kenny *et al*., 1995) possesses a higher affinity for α_{1D} subtype or cloned human α_{1D}-adrenoceptor compared to the other subtypes and this receptor is relatively resistant to alkylation by CEC, and has a low affinity for 5-MeU and (+)niguldipine (Ford, 1994).

The intrarenal arterial administration of Nit, 5-MeU, CEC and BMY 7378 in the present study did not lead to major changes in the RBF in either the diabetic SHR or nondiabetic SHR groups and direct RNS, exogenous NA, PHE and ME gave dose-related reductions in RBF, without major changes in mean arterial pressure (MAP). The pattern of the vasoconstrictor responses obtained in nondiabetic SHR was similar to that of the diabetic SHR, but the magnitude of decrease in RBF induced by the agonists in diabetic SHR was almost always greater than in the nondiabetic SHR. This would be consistent with the earlier observations of Owen & Carrier (1979; 1980), who demonstrated that the aorta of streptozotocin diabetic rats were hyper-responsive to NE. Our findings provide further evidence for this view to include the hyperresponsiveness of the renal vasculature of streptozotocin induced diabetic rats. Furthermore, Dresner *et al*. (1997) were able to show that it was the α_1-, but not α_2-, adrenoceptor mediated vasoconstrictor responses that were enhanced in streptozotocin diabetic rats.

It was evident that CEC did not inhibit the vasoconstrictor responses induced by either RNS or exogenous agonists in diabetic SHR but rather enhanced them. By contrast, in nondiabetic rats the adrenergically induced vasoconstrictions were unaltered, except for the RNS responses, which were enhanced. On the other hand, 5-MeU, Nit and BMY 7378 significantly blunted the vasoconstrictor responses to RNS and the adrenergic agonists in both SHR and diabetic SHR. This would indicate that the postsynaptic α_{1A}- and α_{1D}-adrenoceptor subtypes are involved in mediating the constrictions. The predominant involvement of α_{1A}-adrenoceptor in mediating renal vasoconstrictor responses has been reported previously in other models of hypertension (Sattar & Johns, 1991; 1994a, b; Blue *et al*., 1992; 1995). Furthermore, there has been the suggestion of an involvement of α_{1D}- as well as α_{1A}-adrenoceptors in mediating renal vasoconstriction (Villalobos-Molina & Ibarra, 1996; 1997).

It was of great interest that in the diabetic-SHR, 5-MeU attenuated the renal vasoconstrictor responses to PE and ME, but only had small effects on the renal vasoconstrictor responses to RNS and NA. The possible interpretations of these observations might be that although postsynaptic α_{1A}- and α_{1D}-adrenoceptors in these two groups of rats were involved, a greater component of presynaptic α_{1B}-adrenoceptors were also contributing to offset the postsynaptic responses to exogenously and endogenously released NA, especially in the diabetic SHR. This view was supported to some extent by the observations that when CEC was used in these diabetic SHR, it enhanced the vasoconstrictor responses to both RNS and the other adrenergic agonists, while in nondiabetic SHR only the RNS induced responses were greater while NA, PE and ME induced responses were unaltered. Together, these findings are compatible with a greater participation of presynaptic α_{1B}-adrenoceptors to attenuate α_{1A}-adrenoceptor mediated effects in this model of diabetes and hypertension, which would be supported by an earlier report (Schwartz & Eikenburg, 1988). These authors observed that in the kidneys of NA-treated rats, the enhanced fractional neurotransmitter release that led to vasoconstriction, was a consequence of a reduction in prejunctional α-adrenoceptors. Other possible explanations might be an upregulation of α_{1A}, or an involvement of either presynaptic α_{1B} or α_2-adrenoceptors in determining renal vasoconstriction which, when blocked, removed the presynaptic autoinhibitory feedback, allowing more NA release and a larger postsynaptic response (Li *et al*., 2000).

The α_1-adrenoceptor-induced vasoconstriction appears to be caused both by the release of intracellular calcium and by the trans-membrane influx of intracellular calcium (Cauvin and Malik, 1984; Bylund *et al*., 1994; Zhong & Minneman, 1999). The suggestion that the α_{1A}-adrenoceptor subtype contributed to the adrenergically mediated responses in this diabetic hypertensive model was supported by the finding that the renal vasoconstrictor responses were inhibited after Nit treatment in both nondiabetic and diabetic SHR groups. These results were related to the Ca^{2+} influx dependent

α_{1A}-adrenoceptor activation and compatible with those reported earlier in the hypertensive rats (Sattar & Johns, 1994a).

Alpha$_1$-adrenoceptors are involved in rapid processes such as sequestration and slower processes such as receptor downregulation (Garcia-Sainz, 1993; Cotecchia *et al*., 1995). The slower downregulation of these receptors may be related to pathophysiological processes, which occur in disease states such as cardiac failure and chronic renal failure (Packer, 1992). By contrast, both α_{1A}, and α_{1D}-adrenoceptor subtypes are functionally upregulated in the SHR muscle vascular bed and this may provide some clue for the possible role of α_1-adrenoceptor subtypes in hypertension (Ye & Colquhoun, 1998). In our study, there appears to be little change in the contribution of either α_{1A} or α_{1D} in the renal vasoconstrictor responses as the diabetes developed. On the other hand, the contribution of presynaptic CEC sensitive adrenoceptors (α_{1B} or α_2) seemed to be higher in the diabetic than in the nondiabetic rats. In another study conducted in our lab (unpublished observations), there was a shift in the α_1-adrenoceptor contribution in diabetic Sprague–Dawley rats. In that study, the involvement of α_{1D}-adrenoceptors, which was assessed together with the α_{1A} subtype in normal rats, gradually disappeared as diabetes developed.

The enhancement and leftward shift of the adrenergically induced renal vasoconstrictor responses in the diabetic hypertensive model may have been the result of either impaired neuronal uptake (Garcia *et al*., 1999), super-sensitivity or upregulation of the α_1-adrenoceptors (Tesfamariam & Cohen, 1995), as seen in the responses of the mesenteric arteries of long-term experimental diabetes rat to NA (Jackson & Carrier, 1981). Under these conditions, postsynaptic α_{1B}-adrenoceptors may be downregulated by the adrenergic nerves but the contribution of postsynaptic α_{1A}-adrenoceptors may be enhanced in order to maintain the effectiveness of the α_1-adrenoceptor neuro-transmission system (De Mey, 1997). Owen & Carrier (1980) suggested that super-sensitivity of the rat vasculature to NA in diabetic rats might be due to the alterations in extracellular Ca^{2+} concentration. On the other hand, Kam *et al*. (1996) observed that the streptozotocin-diabetic state did not cause important additional pharmacodynamic changes, despite the morphological alterations in vessels, while there were minimal influences on the vasoconstrictions caused by various agonists (Beenen *et al*., 1996). In addition, the enhancement of vascular reactivity in the hypertensive state was blunted by the simultaneous occurrence of diabetes mellitus (Beenen *et al*., 1996), but this was not apparent from our results.

In conclusion, the findings from this study would suggest that the α_{1A}- and α_{1D}-adrenoceptor subtypes mediate a large component of the adrenergically induced renal vasoconstriction in both nondiabetic and diabetic with hypertensive rats. Moreover, the findings also suggest that there is an involvement of presynaptic α_{1B}-adrenoceptors in mediating the action of the adrenergic agonists based on an overall enhancement of the adrenergic vasoconstrictor responses in the diabetic with hypertensive rats treated with CEC but not in the nondiabetic hypertensive rats. The mechanisms underlying these changes are unclear and require further investigation.

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