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SPECIAL REPORT

Functional evidence of α_{1D}-adrenoceptors in the vasculature of young and adult spontaneously hypertensive rats

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The role of α_{1D}-adrenoceptors in the vasculature of spontaneously hypertensive (SHR) and normotensive Wistar Kyoto rats (WKY), of different ages was assessed in pithed rats by the use of the selective α_{1D}-adrenoceptor antagonist BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro [4.5]decane-7,9-dione dihydrochloride). BMY 7378 displaced the pressor effect of phenylephrine in young pre-hypertensive pithed SHR rats, but produced no effect in young WKY rats (dose ratio of 3.4 and 1.6, respectively), while in adult rats BMY 7378 produced a greater shift in the phenylephrine response curve than in younger animals (dose ratio of 3.2 and 6.2 in WKY and SHR, respectively). The presence of α_{1D}-adrenoceptors in the vasculature of pre-hypertensive rats, suggests its role in the pathogenesis/maintenance of increased blood pressure.

Keywords: α_{1D}-adrenoceptors; BMY 7378; pithed spontaneously hypertensive rats; ageing

Abbreviations: WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; BMY 7378, (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro [4.5]decane-7,9-dione dihydrochloride)

Introduction α_1-Adrenoceptors are involved in smooth muscle contraction and rat aorta seems to be a prototypic tissue for the expression of the α_{1D}-subtype (Hieble *et al*., 1995). It has been reported that vascular α_{1D}-adrenoceptors play a role in pressor responses *in vivo* (Zhou & Vargas, 1996). Vascular contractile responsiveness seems to increase with ageing and this effect may be related to the marked increase in the maximal pressor effect of alpha-1-adrenoceptor stimulation observed in adult pithed rats (Ibarra *et al*., 1997). In addition, α_{1D}-adrenoceptors are the predominant subtype that mediates contraction in aorta, carotid and mesenteric arteries of spontaneously hypertensive rats (SHR) (Villalobos-Molina & Ibarra, 1996). These data suggest that this receptor subtype may be related to the pathogenesis/maintenance of hypertension (Ibarra *et al*., 1998). However, despite the presence of the α_{1D}-adrenoceptor mRNA in many rat tissues the protein is not detectable at the membrane level (Yang *et al*., 1997). This has generated doubts on the functional role of this receptor subtype. However, membranes from rat aorta show an abundant number of this receptor (Deng *et al*., 1996), and its functional role has been evidenced in several arteries with the use of antisense oligonucleotides (Piascik *et al*., 1997). These data suggest that its predominant role is at the vascular level. In an attempt to correlate vascular α_{1D}-adrenoceptors with increase in blood pressure during ageing, we measured the pressor response to phenylephrine in BMY 7378, (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro [4.5]decane-7,9-dione dihydrochloride)-treated, pithed young and adult WKY and SHR rats.

Methods Male WKY and SHR rats of 1 and 6 months of age ($n=43$) were used. All procedures were approved by the Institutional Animal Care and Use Committee. Basal diastolic blood pressure in anaesthetized rats (sodium pentobarbital, 35 mg kg^{-1}, i.p.), were: 93 ± 2 and 100 ± 3 mmHg in 1 month-old WKY and SHR, respectively; while values were: 101 ± 10 and 120 ± 3 mmHg in 6 months-old WKY and SHR, respectively ($P=0.05$). Trachea were cannulated, the animals were pithed and were artificially respired with a Harvard pump (56 cycles min^{-1}; volume: 20 ml kg^{-1}). The vagi were then cut. Catheters were placed in the right femoral vein for drug injection and in the left carotid artery for recording of diastolic blood pressure and heart rate; the cannula placed in the carotid artery was connected to a TXD-300 pressure transducer (Digi-Med, Lexington, KY, U.S.A.).

Diastolic blood pressure was recorded by a BPA-190b blood pressure analyzer (Digi-Med), while the animals were maintained at 37°C.

Once the animals had stabilized for at least 15 min, basal diastolic blood pressure was determined. After collection of this data, rats from both strains and ages received either physiological saline (1 ml kg^{-1}) or BMY 7378 (1 mg kg^{-1}); 15 min later, dose-response curves for phenylephrine (1–1000 μg kg^{-1}; spaced by a factor of $10^{1/2}$), were constructed. Only one agonist dose-response curve was obtained per rat; in all cases, the maximal doses that could be tolerated by the animals were used. All drugs were purchased from Research Biochemicals Int. (Natick, MA, U.S.A.) and were dissolved in physiological saline. Fresh solutions were prepared for each experiment and the doses refer to the free base of substances.

Results are expressed as means$\pm$s.e.mean. Peak changes in diastolic blood pressure evoked by phenylephrine in saline- and antagonist-treated rats of 1- and 6-months of age were determined, and compared using Newman-Keuls' test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations. Where appropriate, the Students' *t*-test was used. ED_{50} values were obtained by non-linear regression. A P value of 0.05 or less was considered statistically significant.

Results Basal values of diastolic blood pressure in WKY of 1- and 6 month-old pithed rats ($n=22$ total) were, 36.0 ± 1.2 and 30.1 ± 1.3 mmHg; while those of SHR ($n=21$ total) were, 36.6 ± 1.2 and 33.7 ± 2.1 mmHg. These values remained unmodified 15 min after injection of either saline or BMY 7378.

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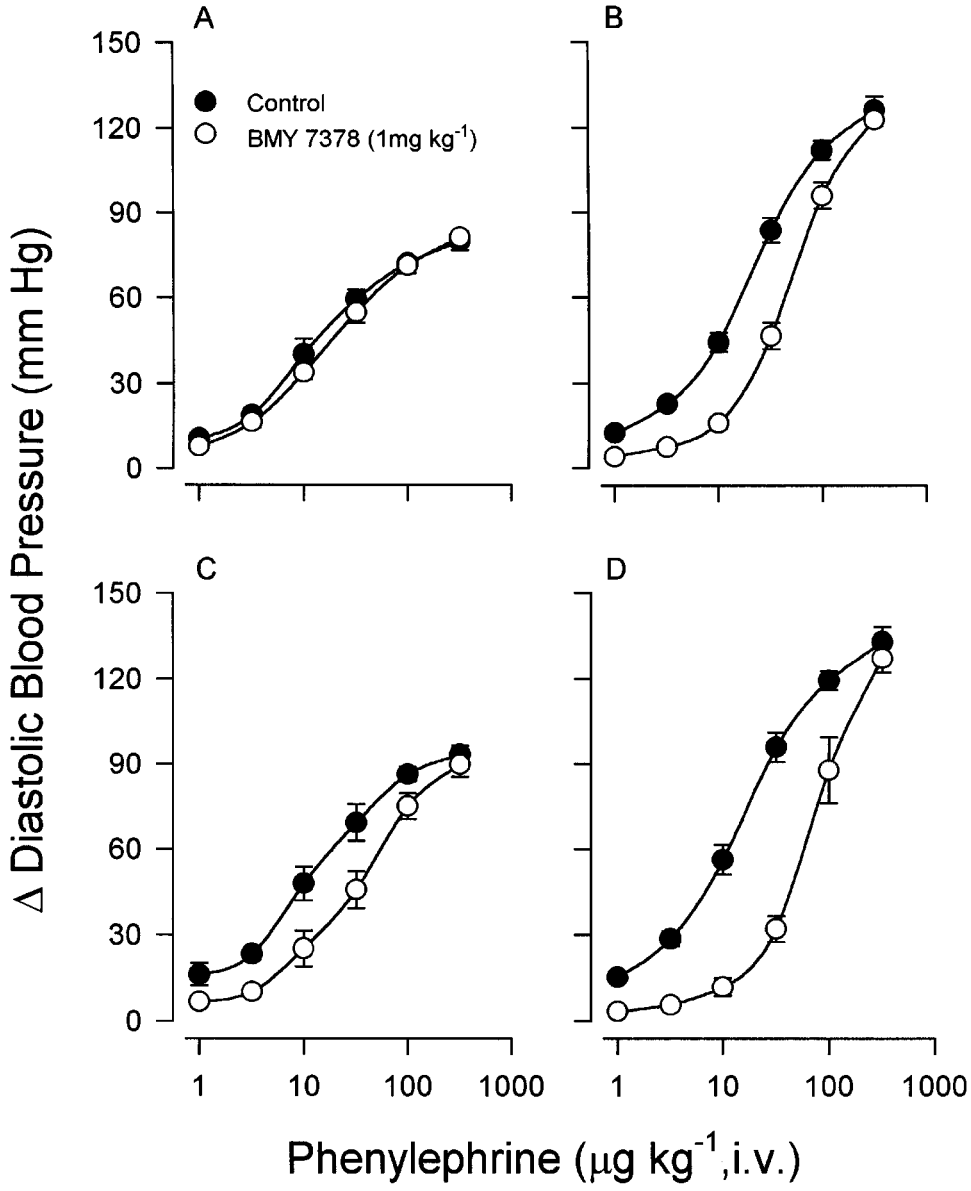

Figure 1 Antagonist action of BMY 7378 in the pressor response to phenylephrine in WKY and SHR rats of different age. Pithed rats of 1 and 6 months of age were subjected to increasing i.v. doses of phenylephrine. (A) WKY, 1 month; (B) WKY, 6 months; (C) SHR, 1 month; (D) SHR, 6 months. Points represent the mean ± s.e.mean of 3–7 rats.

Table 1 Effect of BMY 7378 on phenylephrine-induced pressor effects in 1- and 6-month-old WKY and SHR rats

Group	n	*Agonist ED$_{50}$* (μg kg^{-1}, i.v.)	*E_{max}* (mmHg)	*Dose ratio**
Control				
WKY 1 month	5	10.6 ± 0.8	82 ± 3	–
WKY 6 months	7	17.8 ± 1.5†	127 ± 4	–
SHR 1 months	3	10.2 ± 1.5	99 ± 6^{+}	–
SHR 6 months	7	13.6 ± 1.1	138 ± 4^{+}	–
BMY 7378				
WKY 1 month	5	16.8 ± 2.1#	89 ± 2	1.6
WKY 6 months	5	57.7 ± 7.2†#	130 ± 3	3.2
SHR 1 months	6	34.4 ± 3.4#	99 ± 4†	3.4
SHR 6 months	5	84.4 ± 13.3†#	135 ± 4	6.2

BMY 7378 (1 mg kg^{-1}, i.v.) was administered as a bolus injection and the potency (ED_{50}) and efficacy (E_{max}) estimates for phenylephrine action are presented. Values are the mean ± s.e.mean of *n* experiments. *Ratio of ED_{50} with antagonist/ED_{50} of the control values. †$P<0.05$ vs 1 month; #$P<0.05$ vs control; $^{+}P<0.05$ SHR vs WKY.

The intravenous (i.v.) administration of phenylephrine evoked a dose-dependent increase in diastolic blood pressure, irrespective of age and strain (Figure 1). Phenylephrine evoked a higher maximal pressor effect in young and adult SHR compared to WKY (Table 1). While BMY 7378 only slightly modified phenylephrine action in young WKY rats (Figure 1A; Table 1), significantly shifted to the right the agonist effect in young SHR rats (Figure 1C, Table 1). In contrast, BMY 7378 significantly displaced phenylephrine ED_{50} in both adult WKY and SHR rats (Figure 1B and D; Table 1).

Discussion Our data demonstrate the presence of α_{1D}-adrenoceptors in the vasculature of young, pre-hypertensive SHR rats and its age-related appearance in adult WKY rats; a similar result has been observed with the ageing of the normotensive rat (Ibarra *et al*., 1997). The abundance of this receptor occurring in aorta follows this order SHR > WKY > > Wistar at mRNA, receptor number and functional levels (Stassen *et al*., 1997; Xu *et al*., 1998; Ibarra *et al*., 1998). The presence of α_{1D}-adrenoceptors in the resistance vasculature of pre-hypertensive and hypertensive rats, may indicate that this receptor is involved in the pathogenesis/maintenance of high blood pressure in SHR. Moreover, the maximal pressor effect of phenylephrine was higher in SHR than in WKY rats, suggesting that occurrence of α_{1D}-adrenoceptors could be involved in vascular hyperreactivity.

The facts that BMY 738 blocked α_{1D}-adrenoceptors in isolated arteries of SHR rats (Villalobos-Molina & Ibarra, 1996), and that antisense oligonucleotides decreased aorta contraction to phenylephrine from normotensive rats (Piascik *et al*., 1997), also suggest that this receptor has a major role in the control of vascular function.

Taken together, evidence reported here as well as from the literature points to a possible role of α_{1D}-adrenoceptors in the pathogenesis and/or maintenance of hypertension in SHR rats. Promising areas for future research include the knockout of the α_{1D}-subtype gene in both normotensive and hypertensive animals, the use of antisense oligonucleotides against this subtype as well as the design of new, highly selective α_{1D}-adrenoceptor antagonists with therapeutic potential.

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