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Pre-weaning carvedilol treatment in spontaneously hypertensive rats

Erika I. Boesen[a,*], Gavin W. Lambert[b], Warwick P. Anderson[a], Michelle M. Kett[a]

[a] Department of Physiology, Monash University, Victoria 3800, Australia
[b] Baker Heart Research Institute, Prahran, Victoria, Australia

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Abstract

Hypertension in spontaneously hypertensive rats (SHR) has been permanently abolished by aggressive treatment regimens targeted against the sympathetic nervous system and adrenal medulla, initiated during the pre-weaning period (guanethidine and nerve growth factor antiserum combined with either adrenal demedullation or prazosin treatment). To investigate the components of the sympatho-adrenal system involved, we treated pre-weaning SHR with the combined α_1- and β-adrenoceptor antagonist carvedilol (60 mg/kg/day s.c.; postnatal days 1–21). Carvedilol treatment significantly blocked adrenoceptors during the treatment period, delayed development (eye opening), reduced growth, and reduced arterial pressure and heart rate. However, there was only modest attenuation of the subsequent development of hypertension at 10 weeks of age (mean arterial pressure 129.5 ± 1.8 versus 136.1 ± 1.6 mm Hg in vehicle-treated littermates; $P < 0.05$). Thus pre-weaning carvedilol treatment slightly but significantly attenuated the development of SHR hypertension at 10 weeks, suggesting that the profound antihypertensive effects of pre-weaning sympatho-adrenal ablation are attributable to factors other than α_1- and β-adrenoceptor-mediated effects of catecholamines during this period.

Keywords: Sympathetic nervous system; Andrenoceptor antagonist; Spontaneously hypertensive rat (SHR)

1. Introduction

To date, the only treatment regime that has succeeded in completely preventing hypertension in spontaneously hypertensive rats (SHR) has been neonatal sympathectomy (using guanethidine and nerve growth factor antiserum) combined with either removal of the adrenal medulla (Lee et al., 1991) or treatment with the α_1-adrenoceptor antagonist prazosin from 3 to 6 weeks postnatally (Korner et al., 1993). These approaches appear to abolish hypertension permanently (SHR followed up to 35 weeks of age remain normotensive; Korner et al., 1993; Lee et al., 1991), whereas sympathectomy alone (i.e. without adrenal demedullation or prazosin treatment) only attenuates the extent of hypertension development (Lee et al., 1987). These findings indicate important roles for both circulating catecholamines and the sympathetic nervous system in SHR hypertension. However, the particular sympatho-adrenal mechanisms, such the sympathetic neurotransmitters or adrenoceptor subtypes responsible, are not known.

The striking efficacy of complete sympatho-adrenal ablation when instituted in the neonate implies that effects of the sympathetic nerves soon after birth are critical in SHR hypertension. However, this cannot be stated with certainty since sympathectomy destroys the peripheral sympathetic innervation for life (Lee et al., 1987), and thus the enduring antihypertensive outcome of the treatment could be attributable to ongoing effects of sympathectomy, rather than to effects of sympathectomy specifically during the neonatal period alone. To specifically examine the neonatal period, McCarty and Lee (1996) administered the α_1-adrenoceptor antagonist terazosin to SHR from postnatal day 1 to 21 and found that this early intervention markedly attenuated the severity of hypertension in adulthood. This study suggests therefore that adrenergic influences during the pre-weaning period may play an important role in the severity of the hypertension developed. However, comparable studies investigating the effect of β-adrenoceptor blockade during this period have not been performed.

In the current study therefore, we sought to extend the analysis of the components of the sympatho-adrenal system responsible for the pro-hypertensive effects in SHR by using the combined α_1- and β-adrenoceptor antagonist carvedilol. Carvedilol is a potent α_1-adrenoceptor antagonist (K_B of 11

* Corresponding author. Tel.: +61-3-9905-2555; fax: +61-3-9905-2566.
E-mail address: Erika.Boesen@med.monash.edu.au (E.I. Boesen).

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nM), and has even greater affinity for β-adrenoceptors (K_B of 0.9 nM for β_1 and β_2 subtypes; Ruffolo et al., 1990). SHR pups were treated from postnatal day 1 to 21 and studied at two time-points: at the end of the treatment period to confirm that adrenoceptors were blocked during the treatment period and to record the effects on blood pressure, heart rate and development, and at 10 weeks of age to observe the effect of the pre-weaning treatment on adult blood pressure. We also studied the effect of the pre-weaning carvedilol treatment on peripheral sympathetic innervation, specifically in the kidney due to its importance in long-term blood pressure regulation (Guyton et al., 1972).

2. Methods

This study was approved in advance by the Monash University Physiology Animal Ethics Committee and conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. Pregnant SHR were obtained from the Baker Heart Research Institute (Prahran, Australia) and caged individually. All rats were housed at 22–24 °C, with a 12-h light–dark cycle, with rat chow and water supplied ad libitum. The date of birth (postnatal day 0) was designated as the day the entire litter was first present by 9 am.

2.1. Treatments

On postnatal day 1, all pups were randomly assigned to receive either carvedilol (30 mg/kg s.c.; GlaxoSmithKline, West Sussex, UK) or an equivalent volume of vehicle (3% dimethyl sulfoxide, 97% peanut oil s.c.) twice daily (i.e. 60 mg/kg/day) from postnatal day 1 to 21 inclusive. All pups were weighed daily during the treatment period and the day rat pups first opened their eyes (a developmental landmark) was recorded. Mortality rates were similar in both treatment groups, with 8 out of 37 SHR in the vehicle group and 9 out of 36 in the carvedilol group dying or being euthanised during the study. At 3 weeks of age, the carvedilol treatment was stopped, the pups were weaned and males and females were separated. The female pups were anaesthetized and the effectiveness of the pre-weaning treatment protocol in blocking adrenoceptors was tested as described below. The male pups were not studied at this time point but were instead allowed to grow to 10 weeks of age when blood pressure was measured as described in Section 2.3. This experimental approach was adopted for ethical reasons in order to minimize the total number of animals required in the study.

2.2. Effects of carvedilol treatment at end of treatment period

At the end of the pre-weaning treatment period (i.e. 3 weeks of age), the effectiveness of the carvedilol treatment in blocking α_1- and β-adrenoceptors, and the effect on resting blood pressure and heart rate was studied in the anaesthetized female SHR. SHR had been treated with carvedilol ($n=5$) or vehicle ($n=4$) from birth until the day of use (postnatal day 25 ± 1) with the last dose administered 16.9 ± 0.5 h before the experiment. Once anaesthetized (thiobutabarbitone; 100 mg/kg i.p.; Research Biochemicals International, Natick, USA), SHR were placed on a heating pad to maintain body temperature and catheters inserted into the femoral artery and vein for blood pressure and heart rate recording and intravenous drug administration, respectively. Basal anaesthetized mean arterial pressure and heart rate were recorded before constructing mean arterial pressure and heart rate dose–response curves to the α_1-adrenoceptor agonist phenylephrine (10–80 μg/kg i.v. boluses; Sigma, St Louis, USA) and the β-adrenoceptor agonist isoprenaline (0.1–0.8 μg/kg i.v. boluses; Sanofi Winthrop, Lane Cove, Australia), respectively, to test the effectiveness of carvedilol in blocking adrenoceptors. At the conclusion of the experiment, body length was recorded (nose to base of tail), the kidneys were weighed, and the left kidney immersion-fixed in 10% buffered formalin for immunohistochemical analysis and the right kidney snap frozen in liquid nitrogen and stored at −70 °C for measurement of renal noradrenaline content.

2.3. Effects 7 weeks after cessation of carvedilol treatment (10 weeks of age)

Male SHR were briefly anaesthetized (methohexitone sodium, 50 mg/kg i.p.; Eli Lily, West Ride, Australia) and the caudal artery cannulated for direct arterial pressure monitoring as described previously (Bergstrom et al., 1998; Tomoda et al., 1997). At least 1 h following recovery from anaesthesia, resting conscious mean arterial pressure and heart rate were measured over a period of 20–30 min. An arterial blood sample was then collected for measurement of haematocrit, plasma renin activity (Oliver et al., 1990) and creatinine concentration (Beckman Synchron CX5 Clinical System Analyzer using a modified rate Jaffé method). SHR were then anaesthetized (pentobarbitone sodium, 40 mg/kg i.p.; Rhone Merieux Australia, Australia), the right kidney removed, blotted dry and weighed, with half of the kidney immersion-fixed in 10% buffered formalin for subsequent histological analysis, and the remaining half snap frozen in liquid nitrogen and stored at −70 °C for measurement of renal noradrenaline content. Hearts were removed and the left ventricular weights and body lengths recorded.

2.4. Analysis of renal sympathetic innervation at 3 and 10 weeks

The peripheral sympathetic innervation was studied using paraffin sections (12 μm) from formalin-fixed 3- and 10-week-old SHR kidneys stained for tyrosine hydroxylase, using a mouse anti-rat tyrosine hydroxylase monoclonal primary antibody (DiaSorin, Stillwater, USA) and the avi-

din–biotin complex method. A "blind" observer recorded the vascular level to which the kidney was innervated and ranked the apparent extent of innervation. Renal noradrenaline contents were quantified as described previously (Medvedev et al., 1990).

2.5. Statistics

To compare body weight and dose–response curves for carvedilol- and vehicle-treated SHR, repeated measures analysis of variance (ANOVA) was used (Greenhouse–Geisser correction applied). The Mann–Whitney U (non-parametric) test was used to compare the day rat pups first opened their eyes, and renal tissue noradrenaline content between vehicle- and carvedilol-treated SHR. All other results were analyzed using a one-way ANOVA.

3. Results

3.1. Effects of carvedilol treatment (3 weeks of age)

Anaesthetized basal mean arterial pressures in the 3-week-old SHR were approximately 20 mm Hg lower ($P<0.01$) and heart rates approximately 140 beats/min lower ($P<0.001$) in carvedilol- compared to vehicle-treated SHR (Fig. 1A and B). Testing for adrenoceptor blockade 16.9 ± 0.5 h after the last dose of carvedilol or vehicle was administered, blood pressure and heart rate responses to phenylephrine and isoprenaline, respectively, were markedly attenuated in carvedilol-treated SHR ($P_{Group}<0.01$ and $P_{Dose*Group}<0.01$ for both agents; Fig. 1C and D).

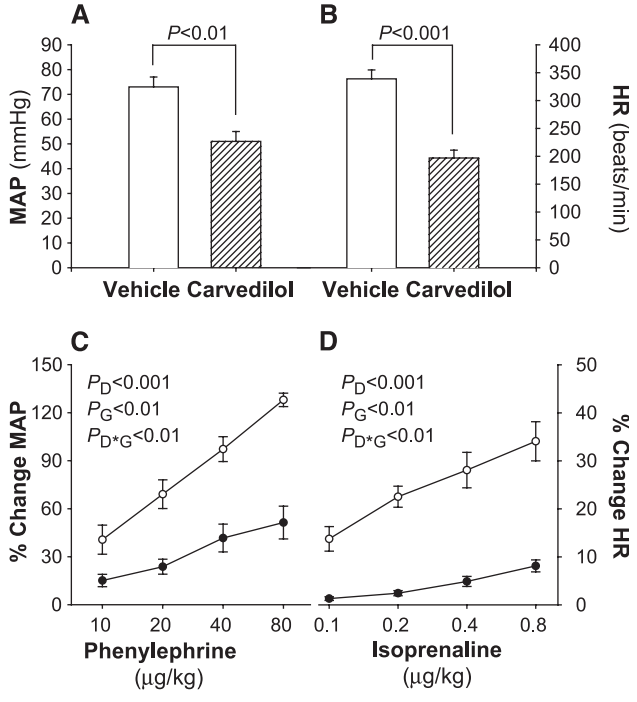

Fig. 1. Basal anaesthetized mean arterial pressure (MAP; A) and heart rate (HR; B) in female vehicle - (open bars; $n=4$) and carvedilol-treated (hatched bars; $n=5$) SHR on postnatal day 25 ± 1 (final dose of carvedilol or vehicle administered 16.9 ± 0.5 h prior to experiment). MAP responses to phenylephrine (C) and HR responses to isoprenaline (D) in vehicle- (open circles) and carvedilol-treated (closed circles) SHR. Data were analyzed by repeated measures ANOVA, testing for an effect of treatment (P_G), dose dependency of responses to agonists (P_D), and an interaction between the two (P_{D*G}). P values were adjusted using the Greenhouse–Geisser correction.

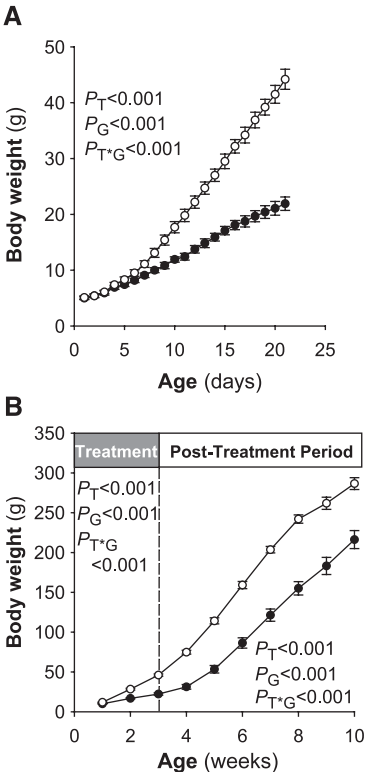

Fig. 2. Body weights of female vehicle- (open circles; $n=12$) and carvedilol-treated SHR (closed circles; $n=12$) from postnatal day 1 to 21 (A), and of male vehicle- (open circles; $n=13$) and carvedilol-treated (closed circles; $n=10$) SHR up to 10 weeks of age (B). Data was analyzed by repeated measures ANOVA, testing for an effect of treatment (P_G) and whether the treatment affected body weight differently between the two groups over time (P_{T*G}). The P values were adjusted using the Greenhouse–Geisser correction.

Carvedilol treatment had a marked effect on the postnatal development of both male and female SHR. The growth rate of carvedilol-treated SHR was significantly lower than vehicle-treated littermates during the pre-weaning treatment period ($P_{Time*Group}<0.001$; Fig. 2A and B) such that at the end of the treatment period body weights of carvedilol-treated rats were 50% lower than vehicle-treated rats (22.3 ± 1.4 and 45.8 ± 1.5 g for male, and 21.9 ± 1.2 and 44.2 ± 1.8 g for female carvedilol- and vehicle-treated SHR, respectively; $P<0.001$). Body lengths of the female carvedilol-treated SHR were 25% shorter than those of vehicle-treated SHR by the end of the treatment period (96 ± 5 and 128 ± 5 mm, respectively; $P<0.01$). Further, the median day on which carvedilol-treated SHR first opened their eyes was 3 days

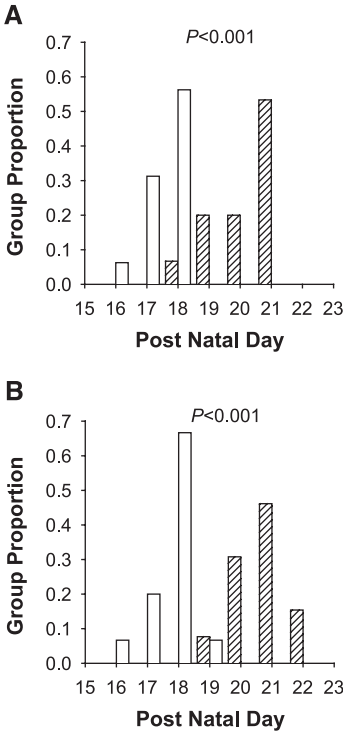

Fig. 3. Proportion of female (A) and male SHR (B) in carvedilol- (hatched bars) and vehicle-treated (open bars) groups to first open their eyes on a particular postnatal day. $n = 13–16$ of each sex in each treatment group. Data was analyzed using the Mann–Whitney *U*-test.

later than vehicle treated littermates for both male and female SHR ($P < 0.001$; Fig. 3A and B).

3.2. Effects 7 weeks after cessation of carvedilol treatment (10 weeks of age)

At 10 weeks of age, mean arterial pressure of carvedilol-treated SHR was approximately 7 mm Hg lower ($P < 0.05$; Table 1) than in vehicle-treated SHR, with no difference detected in heart rate. There were no significant differences between carvedilol- and vehicle-treated SHR in left ventricle-to-body weight ratio, plasma renin activity or creatinine concentration (Table 1). At 10 weeks of age, carvedilol-treated SHR remained significantly smaller than vehicle-treated SHR in terms of body weight and body length ($P < 0.001$; Table 1). The reduced growth rate of carvedilol-treated SHR, apparent during the pre-weaning treatment period, persisted after treatment withdrawal, with carvedilol-treated SHR failing to gain weight at the same rate as vehicle-treated SHR ($P_{Time*Group} < 0.001$; Fig. 2B).

Table 1
Systemic effects of pre-weaning carvedilol treatment at 10 weeks of age

	Vehicle	Carvedilol
Haemodynamics		
Mean arterial pressure (mm Hg)	136.1 ± 1.6	129.5 ± 1.8[a]
Heart rate (beats/min)	343 ± 5	336 ± 6
Other characteristics		
Body weight (g)	302 ± 6	239 ± 7[b]
Body length (mm)	218 ± 2	205 ± 2[b]
Haematocrit	0.46 ± 0.01	0.42 ± 0.01[b]
Plasma renin activity (ng AI/ml/h)	7.42 ± 0.78	6.03 ± 0.86
Plasma creatinine (μM)	36.9 ± 1.5	35.9 ± 1.6
Left ventricle (g)	0.709 ± 0.015	0.575 ± 0.017[b]
Left ventricle: body weight ($\times 10^{-3}$)	2.35 ± 0.02	2.41 ± 0.03

Values represent mean ± S.E.M. for $n = 9–13$ in each group.
[a] $P < 0.05$.
[b] $P < 0.001$.

Table 2
Kidney weights and noradrenaline levels at 3 and 10 weeks

	Vehicle	Carvedilol
Three weeks of age		
Average kidney weight (g)	0.302 ± 0.015	0.165 ± 0.012[a]
Renal noradrenaline (ng/g tissue)	509 (374–746)	1082 (665–1741)[b]
Total kidney noradrenaline (ng)	176 (97–182)	179 (152–290)
Ten weeks of age		
Right kidney weight (g)	1.511 ± 0.049	1.288 ± 0.051[a]
Renal noradrenaline (ng/g tissue)	321 (237–684)	388 (262–916)
Total kidney noradrenaline (ng)	499 (322–866)	540 (359–999)

Values represent mean ± S.E.M. for kidney weights and median with range given in brackets for noradrenaline levels. $n = 6–7$ in each group for female rats at 3 weeks and $n = 10–11$ in each group for male rats at 10 weeks.
[a] $P < 0.001$.
[b] $P < 0.01$.

3.3. Renal sympathetic innervation

Routine histological analysis of kidney sections revealed no gross morphological difference between carvedilol- and vehicle-treated SHR at 3 or 10 weeks of age, however the kidneys from 3-week-old carvedilol-treated SHR were approximately half the size of those from vehicle-treated SHR (Table 2). Immunohistochemical staining of transverse sections from kidneys of 3- and 10-week-old SHR revealed nerves containing tyrosine hydroxylase in all sections studied, being readily identifiable in the perivascular region of intrarenal arteries. There were no discernable differences in the extent of renal innervation between the two groups at either 3 or 10 weeks. Tissue noradrenaline levels expressed per gram of kidney were significantly greater in carvedilol-treated SHR compared to vehicle-treated SHR at 3 weeks ($P < 0.01$; Table 2), but not at 10 weeks of age (Table 2).

4. Discussion

Several approaches have been taken to investigate the role of the sympathetic nervous system in SHR hypertension. The most striking results were obtained sing neonatal sympathectomy combined with either removal of the adrenal medulla (Lee et al., 1991) or treatment with the α_1-adrenoceptor antagonist prazosin from 3 to 6 weeks postnatally (Korner et al., 1993). Completely preventing

hypertension well into adulthood (Korner et al., 1993; Lee et al., 1991), these interventions indicate that the sympathetic nervous system and circulating catecholamines may both play major roles in SHR hypertension.

Investigating the importance of α_1-adrenoceptors in the neonatal/pre-weaning period, McCarty and Lee (1996) found that pre-weaning treatment with terazosin, an agent with primarily α_1-adrenoceptor antagonist properties, produced adult SHR blood pressures approximately midway between that of normotensive Wistar–Kyoto rats and untreated SHR, suggesting a key role for α_1-adrenoceptors in the development of hypertension. Our study has extended the investigation of the adrenergic mechanisms involved in SHR hypertension by blocking β-adrenoceptors in addition to α_1-adrenoceptors during the pre-weaning period using carvedilol. We found that carvedilol treatment during this period in SHR only mildly attenuated hypertension, despite marked α- and β-adrenoceptor receptor antagonism and effects on development.

Our findings of a minimal effect on hypertension with carvedilol contradict the earlier findings of McCarty and Lee using terazosin (McCarty and Lee, 1996). The small effect of carvedilol on adult blood pressure does not appear to be due to inadequate adrenoceptor blockade as we verified that extensive α- and β-adrenoceptor receptor antagonism was achieved using our treatment protocol. The addition of β-adrenoceptor blockade may have counteracted the antihypertensive actions of α_1-adrenoceptor blockade, by some unknown mechanism. However, heart rate was lowered by carvedilol treatment, and since β-adrenoceptor antagonism would be expected to inhibit renin release, it seems more likely that β-adrenoceptor antagonism would augment rather than oppose an antihypertensive effect mediated by blockade of α_1-adrenoceptors. Further, in a study by Lee et al. (1992), continuous β-adrenoceptor blockade from gestation to adulthood attenuated hypertension development in male SHR, although, unlike in our study, the adult rats were still receiving the β-adrenoceptor antagonist at the time of blood pressure measurement.

We suggest that the most likely explanation for the difference in effectiveness of carvedilol and terazosin in attenuating SHR hypertension lies in the affinity of terazosin for the α_{2B}-adrenoceptor in addition to the α_1-adrenoceptor (Hancock et al., 1995). Central α_{2B}-adrenoceptors are thought to be sympathoexcitatory (Gavras et al., 2001), therefore antagonism of these receptors resulting in reduced sympathetic outflow may be partly responsible for the ability of pre-weaning terazosin to attenuate hypertension. Our recent finding that pre-weaning treatment with the α_1-adrenoceptor antagonist doxazosin had no effect on adult blood pressure of SHR (Boesen et al., 2003) supports the hypothesis that the effects of terazosin may not be solely due to α_1-adrenoceptor antagonism.

These findings suggest that pharmacological blockade of adrenoceptors during the pre-weaning period is less effective in abolishing hypertension than complete sympathoadrenal ablation and thus may provide insight into the mechanisms involved in SHR hypertension. One salient difference between the two approaches is that neonatal sympathectomy results in a complete absence of peripheral sympathetic nerves (Lee et al., 1987), whereas sympathetic nerves were indeed still present in kidneys of our carvedilol-treated SHR. Therefore complete sympatho-adrenal ablation would remove catecholaminergic tone for much longer than would pre-weaning carvedilol treatment. Thus, while the findings of the current study suggest that α_1- and β-adrenoceptors do not play a critical role in SHR hypertension during the first 3 weeks postnatally, a role for catecholaminergic influences at a later age cannot be ruled out.

Although α_1- and β-adrenoceptors were blocked during carvedilol treatment, unlike sympathectomized SHR, SHR in the current study would have been exposed to sympathetic co-transmitters such as neuropeptide Y (NPY) and ATP during and after carvedilol treatment. These co-transmitters have several potentially pro-hypertensive effects including vasoconstriction and mitogenic effects (Erlinge et al., 1993; Michel and Rascher, 1995). Further, increased NPY immunoreactivity has been reported in vessels of SHR (Fan et al., 1995). The role of sympathetic co-transmitters in SHR hypertension thus merits further investigation.

It is possible that the modest attenuation of hypertension following pre-weaning carvedilol treatment is attributable to a general delay in development of these animals. The rapid rise in body weight between the ages of approximately 4 and 10 weeks temporally coincides with a rapid rise in blood pressure of male SHR (Burrell et al., 1995). Since both the rise in body weight of the carvedilol-treated SHR and eye opening was delayed, it is possible that other aspects of maturation, including those that are responsible for the rise in blood pressure, were similarly delayed. Thus the development of hypertension may have been merely delayed rather than truly attenuated by the carvedilol treatment.

It is not clear why carvedilol had such an effect on growth and development of SHR. It is possible that nutritional status was altered in the carvedilol-treated pups. However, since it was not feasible to measure the milk intake of the pups, this remains unknown. Previous studies where other adrenoceptor antagonists have been administered to SHR during the pre-weaning period have not shown any difference in body weights between groups (Boesen et al., 2003; Lee et al., 1992; McCarty and Lee, 1996). However, growth retardation to a lesser degree than that produced by carvedilol was reported by Korner et al. (1993) in SHR that underwent complete sympatho-adrenal ablation. Independent of its α_1- and β-adrenoceptor antagonist properties, carvedilol also has a direct antiproliferative effect on SHR cells in vitro (Ohlstein et al., 1994), but whether this effect was responsible for the growth retardation observed in this study is unknown.

In summary, pre-weaning treatment of SHR with carvedilol reduced blood pressure during treatment and slightly, but significantly attenuated the subsequent development of

hypertension. Thus carvedilol was less effective in preventing or attenuating hypertension development in SHR than as previously reported for complete sympatho-adrenal ablation or pre-weaning treatment with terazosin, but more effective than pre-weaning treatment with doxazosin. Together, these results indicate that the profound antihypertensive effects of complete sympatho-adrenal ablation seen previously cannot be solely attributed to the absence of catecholamines acting via α_1- and β-adrenoceptors at effector organs during the pre-weaning period. Rather, these results suggest that this effect occurs via mechanisms that remain to be identified, and may involve other sympathetic neurotransmitter substances or possibly α_2-adrenoceptors. To date, only those treatments that produce an enduring ablation of the sympathetic nervous system appear to abolish SHR hypertension.

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