Influences of carvedilol treatment on the effects of acetylcholine on regional haemodynamics in the spontaneously hypertensive rat

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ABSTRACT

1. In a previous report, we have shown that vasodilatation induced by acetylcholine is impaired in the kidney and the heart of the spontaneously hypertensive rat (SHR) in vivo. The present investigation was performed to study the influence of oral antihypertensive treatment with carvedilol for 6 to 10 weeks on acetylcholine-induced changes in regional haemodynamics in SHR in vivo. Cardiac output, regional blood flow and vascular resistance in organs of major importance in hypertensive disease, such as the kidney, heart, skeletal muscle, brain and eye, were measured with radioactively labelled microspheres in anaesthetized rats (aged 12–16 weeks).

2. Mean arterial blood pressure was significantly lower in the carvedilol-treated SHR group (156 ± 3 mmHg, n = 17) than in an untreated SHR group (172 ± 6 mmHg, n = 13). Infusion of acetylcholine (2 µg·min⁻¹·kg⁻¹) caused similar significant reductions in blood pressure in the two groups (−13 ± 1% and −14 ± 2%). However, acetylcholine induced a significant increase in total peripheral vascular resistance in the carvedilol group (29 ± 10%, P < 0.01), whereas no significant change was observed in the control group (0 ± 11%).

3. Acetylcholine significantly increased renal vascular resistance in the carvedilol group (−62 ± 15%, P < 0.01), but did not change vascular resistance in the control group (−6 ± 6%). In the heart, acetylcholine did not affect vascular resistance in the carvedilol group, but reduced vascular resistance significantly in the control group (−17 ± 8%, P < 0.05). The circulatory changes induced by acetylcholine in the skeletal muscle, brain and ophthalmic circulation did not differ between the groups.

4. In conclusion, the results demonstrate that long-term oral carvedilol treatment in the SHR did not enhance acetylcholine-induced vasodilatation, but instead pronounced renal vasoconstriction was induced by acetylcholine, which could partly be due to a decreased cardiac index.

INTRODUCTION

Soon after the discovery that the vascular endothelial cells have the capacity to synthesize and release a vasodilating factor later identified as nitric oxide (NO) [1,2], it was hypothesized that dysfunction of the vascular endothelium could be of pathophysiological importance for the development of vascular diseases such as hypertension. Studies in vitro using isolated rings of thoracic aorta, coronary and carotid arteries have demonstrated

Key words: acetylcholine, blood flow, carvedilol, haemodynamics, hypertension, spontaneously hypertensive rat.
Abbreviations: CI, cardiac index; MAP, mean arterial pressure; NO, nitric oxide; SHR, spontaneously hypertensive rat; TPRI, total peripheral resistance index.
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an impaired acetylcholine-induced vasodilatation in spontaneously hypertensive rats (SHR) when compared with normotensive rats [3–5]. Furthermore, acetylcholine has been found to induce vasodilatation in vivo in the kidney [6,7] and heart [7] of the normotensive rat, but not in the SHR.

Chronic antihypertensive treatment with angiotensin-converting enzyme inhibitors and calcium-channel antagonists has been found to improve acetylcholine-induced vasodilatation in vitro in different arteries [8–12]. On the other hand, hydralazine did not improve acetylcholine-induced relaxation in large arteries in the SHR, despite a reduction in blood pressure [8,11]. The relatively new antihypertensive agent carvedilol with β- and α1-adrenergic antagonizing effects [13,14] has been found to have a positive effect on acetylcholine-induced relaxation during conditions in which vasculature has been experimentally manipulated, such as during ischaemia or pyrogallol exposure or by increasing blood lipid levels [14–17]. However, acetylcholine relaxation has not been thoroughly studied during carvedilol treatment in the hypertensive rat.

In the present study the radioactively labelled microsphere technique was used to investigate whether long-term oral carvedilol treatment alters the effects of acetylcholine on blood flow and vascular resistance in vivo in the kidney, heart, skeletal muscle, brain and eye of the SHR. In the present experimental set-up the integrated physiological response to acetylcholine, including both effects on the endothelium as well as autoregulatory mechanisms, was evaluated in anaesthetized rats.

METHODS

Male SHRs were obtained from Møllegaard, Denmark. The studies were approved by the Animal Ethics Committee of the University of Uppsala. At 6 weeks of age, the animals were given carvedilol or control pellets until they reached the age when experiments were performed (12–16 weeks). One group of animals (n = 17) was given pellets containing carvedilol, 2400 p.p.m., an oral dose similar to that used by others [18,19]. A group of control animals (n = 13) received pellets without carvedilol. The amount of carvedilol consumed daily was approximately 150–200 mg/kg body weight, calculated by measuring the weight of pellets. The rats were of the same age (12–16 weeks) in both groups when the experiments were performed. At that time the body weight varied from 0.265 to 0.370 kg.

Anaesthesia, operation and microsphere procedure

All animals were anaesthetized intraperitoneally with thiobutabarbitral (120 mg/kg). Body temperature was kept at about 37 °C by a thermistor and heating pad (Atew, Sweden). The operative procedures and determinations and calculations of central and regional haemodynamics have been described in detail previously [7]. The animals were tracheotomized and breathed spontaneously. Both femoral arteries and one femoral vein were cannulated. The right common carotid artery was cannulated and the position in the left ventricle was assessed by pressure registration. The microspheres were given in the left ventricle. At the end of operative procedures, heparin (500 units/kg) was given intravenously to prevent clogging of catheters. Acid–base analysis was performed at the beginning and end of experiments (ABL 300 and Osm 3, Radiometer, Copenhagen, Denmark).

Regional blood flow was determined with 15-μm radioactive microspheres. Spheres labelled with 141Ce and 103Ru were used. The number of spheres injected was 380 000, administered in 0.3 ml of saline. The spheres were injected during 15 s and the reference samples were obtained by constant withdrawal of blood from a femoral artery using a pump at a rate of approximately 0.6 ml/min (P-1, Pharmacia fine chem, Sweden) for 1 min, giving a reference blood sample that was weighed to obtain a precise reference blood flow (in g/min). An additional 0.2 ml of saline was injected after each sphere procedure to compensate for loss of blood volume during the sphere procedure. Cardiac index (CI) was defined as cardiac output per kg of body weight. To calculate total peripheral vascular resistance index (TPRI) the average mean arterial blood pressure (MAP) during the sphere injection was used, obtained from MAP before and 30 s after the beginning of sphere injection. However, the maximal reduction in MAP induced by acetylcholine was obtained after 5 min of infusion. The MAP obtained 5 min after the beginning of acetylcholine infusion was on average 6 and 9 mmHg lower than the average MAP during the sphere procedure in the control and carvedilol group respectively.

At the end of the experiments, tissue samples from both kidneys (whole organs), heart (apical area of left ventricle), biceps muscle, skin (from left foreleg), the left side of the brain (frontal cortex, thalamus and medulla), the hypophysis, ophthalmic tissues (choroid and anterior uvea, whole organs) and lung were dissected for measurement of radioactivity in a gamma spectrometer (Nuclear Chicago Co., IL, U.S.A.). In order to obtain correct activity, background activity and cross-over between energy channels were compensated for in each sphere measurement. Lung tissue was used mainly as a control of shunting of spheres to the right side of the heart. No experiments with very high blood flow in the lung were included. All tissues except the ophthalmic tissues were weighed, blood flow and vascular resistance were related to g/tissue weight (tw), and blood flow was given as g·min⁻¹·g⁻¹·tw. Ophthalmic tissue haemodynamics were
studied in the whole organ and not related to weight, since the low weight would induce an unnecessary loss of precision.

Experimental protocol
An identical protocol was used for both carvedilol-treated animals and control rats. A 15-min stabilizing period was followed by the first sphere injection marked by $^{111}$In. Infusion of acetylcholine (2 µg min $^{-1} $kg $^{-1}$) started 10 min after the first sphere injection using an infusion pump (Sage Instruments). During the sixth minute of acetylcholine infusion, the second sphere marked by $^{103}$Ru was administered. The rate of infusion of acetylcholine was previously chosen after blood pressure dose–response studies to obtain a decrease in blood pressure of 10–15% in a comparison between normotensive and hypertensive rats [7].

Sphere-control experiments
The influence of the sphere procedure was evaluated by giving saline instead of acetylcholine in separate experiments in SHR eight (n = 7). The MAP and CI were reduced by less than 6% between the first and second sphere injections, whereas TPRI tended to increase by 12%. Blood flow decreased less than 15% in the kidneys, biceps muscle and skin. In the heart, cerebral cortex and medulla, blood flow increased by less than 20%. In the choroid, the blood flow was 15±10% higher at the second sphere injection and in the anterior uvea it was 7±13% higher.

Drugs and statistical analysis
The following drugs were used: heparin, diluted to 500 units/ml (Løvens, Denmark); carvedilol and control pellets, obtained from SmithKline Beecham Pharmaceutical Co. (King of Prussia, PA, U.S.A.); acetylcholine chloride (Sigma, St. Louis, U.S.A.), given as an infusion at a constant rate (0.1 ml/min). Radioactively labelled microspheres ($^{141}$Ce, $^{103}$Ru) were purchased from Dupont (Wilmington, U.S.A.) in normal saline containing 0.01% Tween. For comparisons between animal groups Student’s unpaired $t$-test was applied, while the paired $t$-test was used within each group. Values were expressed as means ± S.E.M. and $P < 0.05$ was regarded as significant.

RESULTS

Systemic haemodynamics
MAP and heart rate at baseline were significantly lower in the carvedilol-treated group compared with the control group (10% and 17% respectively), whereas CI was similar in the two groups (Table 1). At baseline, TPRI tended to be lower in the carvedilol group, but this difference was not significant (Table 1). Acetylcholine significantly reduced MAP by 13±1% in the carvedilol group and by 14±2% in the control group ($P < 0.001$), but did not affect heart rate. In the carvedilol-treated group, acetylcholine induced a significant decrease in CI (25±6%, $P < 0.001$), while TPRI increased significantly (29±10%, $P < 0.01$). However, in the control group, neither CI, nor TPRI, was significantly affected by acetylcholine (−3±8% and 0±11% respectively). The change in CI was significantly different between the groups (P < 0.05).

Regional haemodynamics
In both the left and right kidney, baseline blood flow tended to be higher and vascular resistance tended to be lower in the carvedilol group compared with the control group. In the left and right kidney, acetylcholine decreased renal blood flow (−37±5% and −38±5% respectively) and increased vascular resistance (±60±15% and ±62±15% respectively) in the carvedilol-treated group only (Table 2, Figure 1). Both of these effects were significantly different between the groups.

In the heart, acetylcholine tended to increase blood flow in the control group only. This response was significantly different from the response found in the carvedilol group. Vascular resistance was significantly reduced by acetylcholine in the control group (Figure 1 and Table 2), a significantly different response to that seen in the carvedilol-treated group.

In the biceps muscle, acetylcholine did not induce any significant changes in blood flow or vascular resistance in either group. In the skin, acetylcholine administration significantly decreased blood flow in the carvedilol and control groups (−32±8% and −30±8% respectively) and vascular resistance increased significantly (±58±20% and ±47±17% respectively, Table 2).

In the cerebral circulation (Figure 2) acetylcholine did not significantly change blood flow in the cortex and thalamus in either group, but induced small significant reductions in the medulla (from 0.74±0.05 to 0.62±0.04 g min $^{-1} $g $^{-1}$ tw) and in the hypophysis (from 0.96±0.14 to 0.60±0.08 g min $^{-1} $g $^{-1}$ tw) in the carvedilol group. Vascular resistance did not change significantly in any of the cerebral regions studied during acetylcholine infusion, except for in the cortex in the untreated group (−18±7%).

In contrast to the cerebral circulation, acetylcholine induced a significant increase in blood flow in the choroid in the carvedilol group (from 0.036±0.05 to 0.052±0.005 g/min) and in the control group (from 0.047±0.006 to 0.084±0.013 g/min) (Figure 2), while vascular resistance decreased by 34±8% and 40±9% respectively, indicating vasodilatation in both groups. In the anterior uvea, acetylcholine did not induce any significant haemodynamic changes.
Acid–base analysis
Acid–base analysis at the end of each experiment showed no major differences between the two groups. Arterial oxygen saturation was 94 ± 1% in the carvedilol-treated group and 92 ± 1% in the untreated group. $P_{CO_2}$ was 4.9 ± 0.2 kPa and 5.4 ± 0.3 kPa, and standard bicarbonate was 21.1 ± 0.3 mmol/l and 20.7 ± 0.8 mmol/l, in the carvedilol-treated and control groups respectively.

DISCUSSION
The present study showed that long-term oral treatment with carvedilol reduced blood pressure and heart rate in the SHR, but no evidence of improved acetylcholine-induced vasodilatation was found in any of the vascular beds. On the contrary, a significant vasoconstriction in the kidney occurred during acetylcholine administration in the carvedilol-treated group. In the present study long-term oral carvedilol treatment tended to lower TPRI, whereas CI was similar in the groups resulting in a reduction in blood pressure, as also reported by others [20–23].

Carvedilol has previously been shown to have protective haemodynamic effects in the kidney of the SHR [13,14,24] which is supported by the observed tendency towards decreased renal vascular resistance in the present study. A reduction in vasodilatory capacity during stimulation with acetylcholine has previously been reported in isolated perfused kidneys, as well as in vivo, in untreated SHRs [6,7,25]. However, our current data do not support the hypothesis that carvedilol treatment augments acetylcholine-induced renal vasodilatation in hypertensive rats. The large decrease in blood flow in the kidneys during acetylcholine infusion in the carvedilol group can partly be explained by the decrease in CI.
Carvedilol and acetylcholine in hypertensive rats

Figure 1  Effects of acetylcholine (Ach) on myocardial and renal blood flow and vascular resistance in carvedilol-treated SHRs (shaded bars) or untreated controls (open bars)

Values are means and S.E.M. Statistical significance: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ within groups; †$P < 0.05$, ††$P < 0.01$ and †††$P < 0.001$ between groups.

Figure 2  Effects of acetylcholine (Ach) on cerebral and ophthalmic blood flow in carvedilol-treated SHRs (shaded bars) or untreated controls (open bars)

Values are means and S.E.M. Statistical significance: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ within groups.

which reflects a reduced sympathetic activation during acetylcholine infusion in the presence of a $\beta$-blocking agent. The acetylcholine-induced rise in vascular resistance in the kidneys is likely to be one of the major contributions to the increase in total vascular resistance in the carvedilol-treated group, since no other examined tissue showed similar vasoconstriction and blood flow to the kidneys represents a main part of the CI as we have previously shown [26].

Carvedilol has been shown to be cardioprotective in several species reducing left ventricular hypertrophy and experimentally induced myocardial infarction size [13,14,19,27,28]. However, in the present study, no beneficial effect on acetylcholine-mediated myocardial vasodilatation was seen.

Carvedilol increased blood flow to the skin in the normotensive rat [29], but in the present study, similar resting blood flow was found in both groups, both in the skin and the biceps muscle. Impaired acetylcholine-induced vasodilatation has been found in the femoral artery [6], and in our previous study in vivo, acetylcholine induced a reduction of vascular resistance in skeletal muscle in the normotensive rats, but not in the SHR [7]. However, in the present study, carvedilol did not improve acetylcholine-mediated vasodilatation in the skeletal muscle.

Oral carvedilol treatment in stroke-prone SHRs has been shown to be protective during brain ischaemia [13,14]. No general impairment in the acetylcholine response was previously found in the cerebral circulation of the SHR using the microsphere method [7], and carvedilol treatment did not change the effect of acetylcholine on cerebral blood flow.

Non-selective $\beta$-adrenoceptor blocking agents such as timolol, used as topical treatment for glaucoma, might reduce blood flow in the retina [30,31]. In the present study carvedilol did not affect blood flow or vascular resistance, neither in the choroid, nor in the anterior uvea. This might possibly be due to the $\alpha$-antagonizing effects of carvedilol. In the uveal circulation, only the blood flow to the posterior uvea, the choroid, was significantly increased by acetylcholine and this was not altered by carvedilol.

Oral carvedilol treatment (120 mg/kg) improved acetylcholine-induced relaxation in mesenteric artery rings in vitro in the stroke-prone SHR [22]. However, this study was performed in vitro in a different substrain using a vascular bed not examined in the present study. It is known that the number of acetylcholine receptors involved in the NO response varies markedly between different vascular beds, explaining some of the heterogeneity of results from different vessels [32]. Our previous study supports a heterogeneity in the response to acetylcholine between vascular beds in vivo [7].

In our in vivo model acetylcholine is given in order to induce vasodilatation by means of endothelial NO release. However, as the baroreflexes are intact, cardiac output would normally increase in order to avoid hypotension. This response was seen in the normotensive rat [7]. Thus, the present in vivo model is not exclusively evaluating endothelial function and a change in cardiac output induced by baroreflexes has to be taken into account.
account when interpreting the results. Acetylcholine might theoretically have a negative inotropic and chronotropic action on the heart as it is a parasympathetic neurotransmitter. However, no such action was detected in our previous study, neither in the normotensive nor hypertensive rat [7], but it cannot be excluded that the vagal effect of acetylcholine could be revealed during β-blockade explaining the reduction in CI. On the other hand, acetylcholine has been shown to release vasoconstrictive factors blunting the action of NO in the SHR [3]. During β-blockade acetylcholine might result in a further release of such constricting factors, being most prominent in the kidneys and resulting in an increased afterload and thereby a decrease in CI. However, the decrease in CI found in the carvedilol group could also contribute to the calculated increase in TPRI.

In conclusion, in the present in vivo model in the SHR, long-term oral carvedilol treatment reduced blood pressure, but no evidence for an enhanced acetylcholine-induced vasodilatation was found. On the other hand, in the carvedilol-treated rats, acetylcholine induced a pronounced renal vasoconstriction, as well as an increase in TPRI. However, some of the reduction in renal blood flow could be due to a decrease in CI.

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