Activation of Capsaicin-Sensitive Sensory Neurons by Carvedilol, a Nonselective β-Blocker, in Spontaneous Hypertensive Rats

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ABSTRACT

We performed a study in spontaneous hypertensive rats (SHR) to determine whether carvedilol, a nonselective β-adrenoceptor antagonist, activates capsaicin-sensitive sensory neurons (CSSNs), thereby promoting the release of calcitonin gene-related peptide (CGRP), a neuropeptide with an important role in maintenance of cardiovascular homeostasis. Carvedilol given intravenously at a dose of 0.3 mg/kg transiently decreased the mean arterial blood pressure (MABP) and increased renal tissue blood flow with increases in CGRP levels in plasma and kidney. These effects induced by carvedilol were not seen in animals pretreated with capsazepine, an antagonist of capsaicin. Although 1.0 mg/kg carvedilol markedly decreased MABP, it neither increased renal tissue blood flow nor CGRP levels in plasma and kidney. These observations strongly suggested that the low dose of carvedilol might activate CSSNs in SHR to increase the release of CGRP, thereby decreasing blood pressure with an increase in renal tissue blood flow. The effects induced by carvedilol seemed to be mediated by its β₂-adrenoceptor blockade activity.

Capsaicin sensitive sensory neurons (CSSN) are nociceptive neurons that can be found in many tissues within the lining epithelia, around blood vessels, and associated with nonvascular smooth muscle and the myocardium of the atria (Maggi and Meli, 1988). These sensory neurons release calcitonin gene-related peptide (CGRP) on stimulation with various stimuli such as low pH, noxious heat, and proinflammatory cytokines such as tumor necrosis factor-α (Opree and Kress, 2000). CGRP, a polypeptide containing 37 amino acids, has been shown to bind to receptors found in the heart and vessels, thereby exerting various effects to maintain cardiovascular homeostasis (Bell and Macdermott, 1996). CGRP exerts a potent vasorelaxant effect by increasing the synthesis and release of nitric oxide in endothelial cells (Samuelson and Jernbeck, 1991). In addition, CGRP has been shown to possess positive inotropic and chronotropic effects, antiarrhythmic effects, and antilipid oxidation (Franco-Cereceda et al., 1987). Because of such effects, CGRP has been used to treat the patients with congestive heart failure (CHF) and, as a consequence, hemodynamic conditions of patients with CHF were significantly improved (Shekhar et al., 1991). These observations further suggest that pharmacological activation of CSSN might be useful in the treatment of CHF.

Carvedilol, a nonselective β-adrenoceptor antagonist with α₁-adrenoceptor blockade activity, has been used as a therapeutic agent for hypertension and CHF (Nichols et al., 1991; Cleland, 1997). Carvedilol has been shown to improve the outcome of patients with severe CHF in a randomized, double-blind, placebo-controlled trial (Packer et al., 2001). Furthermore, a multicenter, double-blind, randomized parallel group trial demonstrated that mortality in patients with mild to severe CHF was significantly lower with carvedilol than with metoprolol, a selective β₁-adrenoceptor antagonist (Pole-Wilson et al., 2003). Although the antioxidant activity of carvedilol is considered to be one explanation for its superior effect in the treatment of CHF (Cleland and Swed-
berg, 1996), other unknown effects might underlie the therapeu
tic usefulness.

Bolles et al. (2003) recently demonstrated that β2-adrenocep
tor stimulation resulted in inhibition of CGRP release from senso
ry neurons in vitro, suggesting that carvedilol might increase CGRP release via inhibition of β2-adrenocep
tor activation when the sympathetic nervous system is acti
vated. Thus, we hypothesized that carvedilol might increase
the release of CGRP from CSSN, thereby exerting beneficial
effects in the treatment of hypertension and CHF. To exam
ine this hypothesis, we attempted to determine in the present
study whether carvedilol affects hemodynamic conditions in
spontaneous hypertensive rats (SHR) by stimulating CSSN.
Effects of carvedilol were compared with those of prazosin, a
selective α1-adrenoceptor antagonist; bisoprolol, a selective
β1-adrenoceptor antagonist; and ICI 118,551, a selective β2-
adrenoceptor antagonist, to determine whether the β2-adre
noceptor blockade activity of carvedilol might be important
for enhancement of CGRP release in SHR.

Materials and Methods

Reagents. Carvedilol was kindly provided by Daiichi Pharmaceu
tical Co. (Tokyo, Japan). Bisoprolol was kindly provided by Tanabe
Seiyaku Co. (Tokyo, Japan). Capsaicin, capsazepine (CPZ) (a va
nilloid receptor-1 antagonist), and prazosin hydrochloride (a selec
tive α1-adrenoceptor antagonist) were purchased from Sigma-Al
drich (St. Louis, MO). ICI 118,551 (a selective β2-adrenoceptor
antagonist) was purchased from Tocris Cookson Inc. (Bristol, UK).
All other reagents were of analytical grade.

Administration of Various Agents. Carvedilol, capsazepine,
and capsaicin were dissolved in 10% Tween 20/10% ethanol with
saline. Carvedilol (0.3 and 1.0 mg/kg) was injected i.v. as described
previously (Hashimoto et al., 1991). Bisoprolol (0.3 mg/kg), prazosin
(0.3 mg/kg), and ICI 118,551 (0.25 and 1.0 mg/kg) were dissolved in
saline and injected i.v. as described previously (Smith et al., 1992,
Aidonidis et al., 1994; Quevedo et al., 1999). Capsazepine (15 mg/kg)
was injected s.c. 30 min before administration of various agents as
described previously (Perkins and Campbell, 1992). Capsaicin (1.0 mg/kg)
was injected s.c. as described previously (Erin et al., 2000).

Results

Effects of Carvedilol and/or Capsazepine on Mean Arterial Blood Pressure (MABP) and Plasma Levels of CGRP in SHR. When administered intravenously, carv
dilol at a dose of 0.3 mg/kg transiently decreased the MABP by
about 40 mm Hg at 15 min after administration (Fig. 1A). Administra
tion of capsazepine, a vanilloid receptor-1 antagon
ist, slightly, but significantly, increased MABP from 30 to
60 min after administration (Fig. 1A). Carvedilol-induced
decrease in MABP was completely antagonized by pretreat
ment with capsazepine (Fig. 1A). Although carvedilol at a
higher dose (1.0 mg/kg) markedly decreased MABP, pretreat
ment with capsazepine did not antagonize this decrease (Fig.
1B). Plasma levels of CGRP were significantly increased at
15 min after administration of 0.3 mg/kg carvedilol, but these
levels were not increased by administration of a higher dose of
 carvedilol (1.0 mg/kg) (Fig. 2). Plasma levels of CGRP were

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Determination of Plasma Calcitonin Gene-Related Peptide Level. Plasma levels of CGRP were determined in animals by modi
fication of the methods as described previously (Gangula et al.,
2000). Plasma samples were acidified with 10% of trifluoroacetic acid
(TFA) (450 μl plasma with 50 μl of TFA) and incubated on ice for 20
min. Acidified samples were centrifuged at 6000g for 15 min at 4°C,
and the supernatant was retained. The pellets were then treated
with 300 μl of 10% TFA, resuspended, and centrifuged as described
above. CGRP was extracted from the supernatant by using reverse phase C18 columns (Amersham Biosciences UK, Ltd., Buckingham
shire, Little Chalfont, UK). Columns were prepared by washing with
5 ml of methanol, followed by 10 ml of water before use. The super
natant was applied onto the column, followed by washing with 20 ml
of 0.1% TFA. CGRP was eluted with 3 ml of 60% acetonitrile in 0.1% TFA, and the solvent was evaporated under a stream of nitrogen gas.
The concentration of CGRP was assayed by using a specific enzyme
immunoassay kit (SPI-BIO, Massey Cedex, France). The sensitivity
of the CGRP assay was 10 pg/ml. The antiserum cross-reacted 100%
of rat α- and β-CGRP according to the manufacturer's data sheet.

Measurement of Renal Tissue Blood Flow. Renal tissue blood flow was measured by laser-Doppler flowmeter (ALP21N; Advance,
Tokyo, Japan), as described previously (Mizutani et al., 2000). After
anesthesia with intraperitoneal administration of sodium pentobarb
ital (50 mg/kg), the right jugular veins of these animals were can
nulated with a PE-10 catheter for continuous infusion of normal saline. The Doppler flowmeter probe was placed on the renal cortex.
Renal tissue blood flow was measured from 30 min before drug
administration. The results are expressed as percentage of initial
levels.
slightly, but significantly, lower in animals given capsazepine at 45 min after administration than those given saline alone (control animals) (Fig. 2). Increases in plasma levels of CGRP seen in animals given 0.3 mg/kg carvedilol were almost completely inhibited by pretreatment with capsazepine (Fig. 2). Plasma levels of CGRP in animals given 0.3 and 1.0 mg/kg carvedilol, but pretreated with capsazepine, were significantly lower than those of control animals (Fig. 2).

**Effects of Prazosin, Bisoprolol, ICI 118,551, Capsaicin, and/or Capsazepine on Mean Arterial Blood Pressure and Plasma Levels of CGRP in SHR.** Intravenous administration of prazosin (0.3 mg/kg), a selective α1-adrenoceptor antagonist, and bisoprolol (0.3 mg/kg), a selective β1-adrenoceptor antagonist, decreased MABP by about 30 and 25 mm Hg at 15 min after administration, respectively (Fig. 3, A and B). However, decreases in MABP induced by prazosin and bisoprolol were not reversed by capsazepine pretreatment (Fig. 3, A and B). Administration of ICI 118,551, a selective β2-adrenoceptor antagonist, at doses of 0.25 and 1.0 mg/kg decreased MABP by about 30 and 80 mm Hg, at 15 min after administration, respectively (Fig. 3, C and D). Although the decrease in MABP induced by 0.25 mg/kg ICI 118,551 was antagonized by capsazepine, that induced by 1.0 mg/kg ICI 118,551 was not (Fig. 3, C and D). Subcutaneous administration of capsaicin (1.0 mg/kg) decreased MABP by about 40 mm Hg at 15 min after administration and this decrease in MABP was completely antagonized by pretreatment with capsazepine (Fig. 3E). MABP of animals given prazosin, bisoprolol, or ICI 118,551 at doses of...
0.25 and 1.0 mg/kg, and capsaicin, but pretreated with capsazepine, were significantly higher than those of control animals (Fig. 3, A–E).

Plasma levels of CGRP were not changed at 15 min after administration of prazosin and bisoprolol (Fig. 4). ICI 118,551 at a dose of 0.25 mg/kg significantly increased plasma levels of CGRP at 15 min after administration, whereas this agent at a dose of 1.0 mg/kg did not (Fig. 4). Subcutaneous administration of capsaicin increased plasma levels of CGRP at 15 min after administration (Fig. 4). Increases in plasma levels of CGRP seen in animals given ICI 118,551 at doses of 0.25 and 1.0 mg/kg as well as capsaicin, but pretreated with capsazepine, were significantly lower than those of control animals (Fig. 4).

**Effects of Carvedilol and/or Capsazepine on Renal Tissue Blood Flow and Renal Tissue Levels of CGRP in SHR.** Intravenous administration of carvedilol at a dose of 0.3 mg/kg significantly increased renal tissue blood flow in SHR, whereas that of carvedilol at a higher dose (1.0 mg/kg) did not (Fig. 5). Renal tissue blood flow was significantly decreased transiently after administration of capsazepine (Fig. 5). Increases in renal tissue blood flow in animals given carvedilol at a dose of 0.3 mg/kg were completely inhibited by pretreatment with capsazepine (Fig. 5). Renal tissue levels of CGRP were significantly increased at 30 min after administration of 0.3 mg/kg carvedilol, whereas they were not increased by administration of 1.0 mg/kg carvedilol (Fig. 6). Increases in renal tissue levels of CGRP in animals given carvedilol at a dose of 0.3 mg/kg were completely inhibited by pretreatment with capsazepine, and renal tissue levels of CGRP in animals given carvedilol at doses of 0.3 and 1.0 mg/kg, but pretreated with capsazepine, were significantly lower than those of control animals (Fig. 6).

**Effects of Prazosin, Bisoprolol, ICI 118,551, Capsaicin, and/or Capsazepine on Renal Tissue Blood Flow and Renal Tissue Levels of CGRP in SHR.** Neither prazosin nor bisoprolol given intravenously affected the renal tissue blood flow (data not shown). Although intravenous administration of ICI 118,551 at a dose of 0.25 mg/kg significantly increased renal tissue blood flow, that of a higher dose of ICI 118,551 (1.0 mg/kg) did not (Fig. 7, A and B). Subcutaneous administration of capsaicin significantly increased renal tissue blood flow (Fig. 7C). Administration of capsaicin at ASPET Journals on June 7, 2017 jpet.aspetjournals.org Downloaded from...
epine significantly decreased renal tissue blood flow (Fig. 7, A–C) and almost completely inhibited increases in renal tissue blood flow induced by 0.25 mg/kg ICI 118,551 and capsaicin (Fig. 7, A and C). Renal tissue blood flow in animals given ICI 118,551 at doses of 0.25 and 1.0 mg/kg and capsaicin, but pretreated with capsazepine, was significantly lower than that of control animals (Fig. 7, A–C).

Renal tissue levels of CGRP in animals given prazosin and bisoprolol were not changed (data not shown). Although renal tissue levels of CGRP in animals given ICI 118,551 at a dose of 0.25 mg/kg were significantly increased, they were not changed in animals given ICI 118, 551 at a dose of 1.0 mg/kg (Fig. 8). Subcutaneous administration of capsaicin increased renal tissue levels of CGRP (Fig. 8). Pretreatment of animals with capsazepine significantly decreased renal tissue levels of CGRP compared with those of control animals (Fig. 8). Renal tissue levels of CGRP in animals given ICI 118,551 at doses of 0.25 and 1.0 mg/kg and capsaicin were significantly lower than those of control animals (Fig. 8).

Discussion

As shown in the present study, intravenous administration of carvedilol transiently, but significantly, decreased MABP in SHR in a dose-dependent manner. Pretreatment with capsazepine completely reversed the decrease in MABP induced by 0.3 mg/kg carvedilol, whereas it did not reverse the decrease in MABP induced by 1.0 mg/kg carvedilol. Although plasma levels of CGRP were increased after administration of 0.3 mg/kg carvedilol, they were not changed by administration of 1.0 mg/kg carvedilol. Pretreatment with capsazepine completely reversed the MABP decrease induced by 0.3 mg/kg carvedilol, whereas it did not reverse the decrease in MABP induced by 1.0 mg/kg carvedilol. These observations strongly suggested that the low dose of carvedilol might increase CGRP release, thereby decreasing MABP in SHR. Because MABP was slightly, but significantly, increased with decreases in plasma levels of CGRP after administration of capsazepine in SHR, CGRP might play a critical role in regulation of MABP in SHR. Consistent with this assumption is a previous report demonstrating that the sensitivity of the vasculature to vasodilation by exogenous CGRP was significantly increased in SHR compared with that in corresponding normotensive Wistar-Kyoto rats (Kawasaki et al., 1990).
Although both prazosin, a selective α₁-adrenoceptor antagonist, and bisoprolol, a selective β₁-adrenoceptor antagonist, decreased MABP in SHR, decreases in MABP induced by these agents were not antagonized by pretreatment with capsazepine. Furthermore, neither of these agents increased plasma levels of CGRP in SHR. ICI 118,551, a selective β₂-adrenoceptor antagonist at doses of 0.25 and 1.0 mg/kg, decreased MABP in SHR, whereas pretreatment with capsazepine only antagonized the decrease in MABP in animals given 0.25 mg/kg ICI 118,551. ICI 118,551 at a dose of 0.25 mg/kg increased plasma levels of CGRP, whereas this agent at a dose of 1.0 mg/kg did not. Administration of capsaicin mimicked the effects induced by low doses of carvedilol and ICI 118,551 in SHR. These observations strongly suggested that the low dose of carvedilol might decrease MABP by increasing CGRP release in SHR and this effect might depend mainly on its β₂-adrenoceptor blockade activity. These observations are consistent with the previous reports demonstrating that β₂-adrenoceptor activation inhibited CGRP release from sensory neurons in vitro superfusion of bovine dental pulp (Bowles et al., 2003).

Both blood flow and tissue levels of CGRP in the kidney of SHR were increased by administration of 0.3 mg/kg carvedilol, whereas they were not increased by administration of 1.0 mg/kg carvedilol as shown in the present study. Both increases in renal tissue blood flow and renal tissue levels of CGRP in animals given 0.3 mg/kg carvedilol were completely antagonized by pretreatment with capsazepine, suggesting that the low dose of carvedilol might decrease renal tissue blood flow by increasing CGRP release in SHR. Pretreatment with capsazepine significantly decreased both renal tissue blood flow and renal tissue levels of CGRP, suggesting that CGRP might play an important role in regulation of renal tissue blood flow in SHR. Neither prazosin nor bisoprolol increased renal tissue blood flow or the renal tissue levels of CGRP as shown in the present study. In contrast, ICI 118,551, only at the dose of 0.25 mg/kg, increased renal tissue blood flow and renal tissue levels of CGRP. Increases in both blood flow and tissue levels of CGRP in the kidney of animals given 0.25 mg/kg ICI 118,551 were completely antagonized by pretreatment with capsazepine. Administration of capsae-
5% with administration of several increase in ejection fraction in patients with CHF was about (land et al., 1996). Consistent with this assumption, the mean sympathetic activation and improve cardiac function (Cleland, 1997) reported that the increase in ejection fraction was more than 9% in patients with CHF treated with carvedilol. These observations strongly suggested that the low dose of carvedilol might increase the renal tissue blood flow by increasing CGRP release from sensory neurons via inhibition of β2-adrenoceptor activation in SHR. Systemic infusion of CGRP in rats has been shown to decrease blood pressure with increases in both renal tissue blood flow and the glomerular filtration rate (Amuchastegui et al., 1994), supporting the assumption described above. However, the reason why inhibition of β2-adrenoceptor activation by high doses of carvedilol and ICI 118,551 did not increase CGRP release in SHR is not known.

In the present study, higher dose of capsazepine (15 mg/kg) was administered intravenously to SHR compared with that used in another study using SHR (Li et al., 2003). Because capsazepine has been shown to block the voltage-activated calcium current in sensory neurons (Docherty et al., 1997), it increases MABP and decreases renal tissue blood flow in SHR by inhibiting CGRP release not only via its competitive antagonism with vanilloid receptor-1 but also by inhibition of calcium influx into sensory neurons.

Treatment of CHF with β-adrenoceptor antagonists is considered advantageous, because these drugs attenuate reflex sympathetic activation and improve cardiac function (Cleland et al., 1996). Consistent with this assumption, the mean increase in ejection fraction in patients with CHF was about 5% with administration of several β-adrenoceptor antagonists other than carvedilol (Packer et al., 2001). However, Cleland (1997) reported that the increase in ejection fraction was more than 9% in patients with CHF treated with carvedilol. CGRP has been shown to possess positive inotropic and chronotropic effects (Franco-Cereceda et al., 1987), and it was demonstrated to inhibit sympathetic nervous activity (Oh-hashi et al., 2001). Plasma levels of CGRP were markedly increased in patients with untreated CHF and these levels were decreased after treatment (Ferrari et al., 1991), suggesting that activation of CSSNs might be a compensatory response that maintains cardiovascular homeostasis in patients with CHF. Consistent with this hypothesis is the observation that infusion of CGRP in patients with CHF significantly improved their hemodynamic condition (Nichols et al., 1991). These observations raise the possibility that therapeutic effects of carvedilol in patients with CHF can at least partly be mediated by CGRP released from CSSN through inhibition of β2-adrenoceptor activation.

Although additional activities of carvedilol such as α-adrenoceptor blockade activity (van Zweiten, 1993), antioxidant activity (Feuerstein et al., 1993), and antiendothelin effects (Massart et al., 1999) may be particularly important for reduction of mortality in patients with severe CHF, the precise mechanisms of the therapeutic effects remain unclear. In addition, Cheng et al. (1999) demonstrated that carvedilol blocked K+ and Ca2+ currents concomitantly in rabbit ventricular myocytes, suggesting that carvedilol might be beneficial in the treatment of ventricular tachyarrhythmias by prolongation of action potential duration with minimal reverse frequency dependence. This activity of carvedilol might also contribute to the beneficial effects in the treatment of CHF. Based on these observations in the present study, it is possible that enhanced activation of CSSN by carvedilol, leading to the release of CGRP, might contribute to ameliorate the pathological conditions of CHF by improving the hemodynamic conditions of CHF. Because this effect of carvedilol might depend on its β2-adrenoceptor blockade activity as shown in the present study, the difference in the therapeutic effects in CHF between carvedilol and metoprolol, a selective β1-adrenoceptor antagonist (Pole-Wilson et al., 2003), might be at least partly explained by the β2-adrenoceptor blockade activity of carvedilol.

References


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