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Activation of capsaicin-sensitive sensory neurons by carvedilol, a non-selective 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 non-selective β-adrenoceptor antagonist, activates capsaicin-sensitive sensory neurons (CSSN), 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 of cavedilol markedly decreased MABP, it neither increased renal tissue blood flow nor CGRP levels in plasma and kidney. Prazosin, a selective α_1-adrenoceptor antagonist, and bisoprolol, a selective β_1-adrenoceptor antagonist, decreased MABP with capsazepine showing no antagonistic action in either cases, and these agents increased neither renal tissue blood flow nor levels of CGRP in plasma and kidney. Both ICI 118,551, a selective β_2- adrenoceptor antagonist, at a dose of 0.25 mg/kg and capsaicin mimicked effects induced by 0.3 mg/kg of carvedilol. Administration of 1.0 mg/kg of ICI 118,551 produced effects similar to those induced by 1.0 mg/kg of carvedilol. These observations strongly suggested that the low dose of carvedilol might activate CSSN 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 appeared to be mediated by its β_2-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 chronotorpic effects, antiarrythmic 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 non-selective β-adrenoceptor antagonist with α_1-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 β_1-adrenoceptor anatagonist (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 Swedberg, 1996), other unknown effects might underlie the therapeutic usefulness.

Bowles et al. (Bowles et al., 2003) recently demonstrated that β_2-adrenoceptor stimulation resulted in inhibition of CGRP release from sensory neurons *in vitro*, suggesting that carvedilol might increase CGRP release via inhibition of β_2-adrenoceptor activation when the sympathetic nervous system is activated. 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 examine 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 α_1-adrenoceptor antagonist; bisoprolol, a selective β_1-adrenoceptor antagonist; and ICI 118,551, a selective β_2-adrenoceptor antagonist, to determine whether the β_2-adrenoceptor blockade activity of carvedilol might be important for enhancement of CGRP release in SHR.

Materials and Methods

Reagents

Carvedilol was kindly provided by Daiichi Pharmaceutical Co. (Tokyo, Japan). Bisoprolol was kindly provided by Tanabe Seiyaku Co. (Tokyo, Japan). Capsaicin, capsazepine (a vanilloid receptor-1 antagonist), and prazosin hydrochloride (a selective α_1-adrenoceptor antagonist) were purchased from Sigma Chemical Co (St. Louis, MO). ICI 118,551 (a selective β_2-adrenoceptor antagonist) was purchased from Tocris Cookson Ltd. (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 intravenously (iv) 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 iv as described previously (Smith et al., 1992, Aidonidis et al., 1994, Quevedo et al., 1999). Capsazepine (15 mg/kg) was injected subcutaneously (sc) 30 min prior to administration of various agents as described previously (Perkins and Campbell, 1992). Capsaicin (1.0 mg/kg) was injected sc as described previously (Erin et al., 2000). Solutions were prepared immediately before the experiments. Each control animal received the vehicle in these experiments. However, because results in control experiments using the vehicle of each solution were not significantly different from those obtained by using saline (data not shown), we used, as a

representative control, the data obtained by using saline in the present study.

Measurement of arterial blood pressure

Male spontaneous hypertensive rats (SHR) (280-320 g) aged 10 weeks were purchased Nihon SLC (Hamamatsu, Japan). The care and handling of the animals were in accordance with the National Institutes of Health guidelines. All experimental procedures described below were approved by the Kumamoto University Animal Care and Use Committee. Animals were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg). The right femoral artery was cannulated with 22 gauge angiocatheter and connected with a strain-gauge transducer (Viggo-Spectramed, Singapore) for measuring the arterial blood pressure continuously as described previously (Uchiba et al., 1996). The data were recorded using a Mac Lab data acquisition system (ADI Diagnostics Inc., Rexdale, Ontario, Canada) in conjunction with a Macintosh 7200 computer (Apple Computers, Cupertino, CA). Anesthesia was maintained by intraperitoneal administration of sodium pentobarbital (10 mg/kg) during the experiment.

Determination of plasma calcitonin gene-related peptide level

Plasma levels of calcitonin gene-related peptide (CGRP) were determined in animals by modification 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 6000 g for 15

min at 4°C and the supernatant was retained. The pellets were then treated with 300 µl of 10% TFA, re-suspended and re-centrifuged as described above. CGRP was extracted from the supernatant by using reverse phase C_{18} columns (Amersham, Buckinghamshire, England). Columns were prepared by washing with 5 ml of methanol, followed by 10 ml of water before use. The supernatant was applied onto the column, followed by washing with 20 ml of 0.1% TFS. 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 crossreacted 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 (ALF21N, Advance, Tokyo, Japan), as described previously (Mizutani et al., 2000). Following anesthesia with intraperitoneal administration of sodium pentobarbital (50 mg/kg), the right jugular veins of these animals were cannulated 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 prior to drug administration. The results are expressed as % of initial levels.

Determination of renal calcitonin gene-related peptide level

Renal levels of calcitonin gene-related peptide (CGRP) were determined in animals by modification of the methods as described previously (Harada et al., 2002). In brief, the kidney was weighed and then homogenized in 3 ml of 2N acetic acid. The homogenates were bathed in 90°C water for 20 min and then centrifuged at 4500 g for 10 min (4°C). CGRP was extracted from the supernatant by using reverse phase C_{18} columns (Amersham, Buckinghamshire, England). Columns were prepared by washing with 5 ml of methanol, followed by 10 ml of water before use. The supernatant was applied onto the column, followed by washing with 20 ml of 0.1% trifluoroacetic acid. CGRP was eluted with 3 ml of 60% acetonitrile in 0.1% trifluoroacetic acid 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 crossreacted 100% of rat α- and β-CGRP according to the manufacturer's data sheet. Results are expressed as μg of CGRP per gram of tissue.

Statistical analysis

Data are expressed as the mean ± S.D. The results were compared using either ANOVA followed by Scheffe's post hoc test or an unpaired *t* test. A level of $p < .05$ was considered statistically significant.

Results

Effects of carvedilol and/or capsazepine on mean arterial blood pressure and plasma levels of CGRP in SHR

When administered intravenously, carvedilol at a dose of 0.3 mg/kg transiently decreased the mean arterial blood pressure (MABP) by about 40 mmHg at 15 min after administration (Fig. 1, A). Administration of capsazepine, a vanilloid receptor-1 antagonist, slightly, but significantly, increased MABP from 30 to 60 min after administration (Fig. 1, A). Carvedilol-induced decrease in MABP was completely antagonized by pretreatment with capsazepine (Fig. 1, A). Although carvedilol at a higher dose (1.0 mg/kg) markedly decreased MABP, pretreatment with capsazepine did not antagonize this decrease (Fig. 1, B). Plasma levels of CGRP were significantly increased at 15 min after administration of 0.3 mg/kg of carvedilol, but these levels were not increased by administration of a higher dose of cravedilol (1.0 mg/kg) (Fig. 2). Plasma levels of CGRP were 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 of 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 of 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

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Intravenous administration of prazosin (0.3 mg/kg), a selective α_1-adrenoceptor antagonist, and bisoprolol (0.3 mg/kg), a selective β_1-adrenoceptor antagonist, decreased MABP by about 30 and 25 mmHg 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 β_2-adrenoceptor antagonist, at doses of 0.25 and 1.0 mg/kg decreased MABP by about 30 and 80 mmHg, at 15 min after administration, respectively (Fig. 3, C and D). Although the decrease in MABP induced by 0.25 mg/kg of ICI 118,551 was antagonized by capsazepine, that induced by 1.0 mg/kg of ICI 118,551 was not (Fig. 3, C and D). Subcutaneous administration of capsaicin (1.0 mg/kg) decreased MABP by about 40 mmHg at 15 min after administration and this decrease in MABP was completely antagonized by pretreatment with capsazepine (Fig. 3, E). 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, while 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, while 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 was 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 of carvedilol, while they were not increased by administration of 1.0 mg/kg of 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. 7, C). Administration of capsazepine 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 of 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).

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Discussion

As shown in the present study, intravenous administration of carvedilol transiently, but significantly, decreased MABP in SHR in a dose dependent fashion. Pretreatment with capsazepine completely reversed the decrease in MABP induced by 0.3 mg/kg of carvedilol, while it did not reverse the decrease in MABP induced by 1.0 mg/kg of carvedilol. Although plasma levels of CGRP were increased after administration of 0.3 mg/kg of carvedilol, they were not changed by administration of 1.0 mg/kg of carvedilol. Pretreatment with capsazepine completely reversed the MABP decrease induced by 0.3 mg/kg of carvedilol, while it did not reverse the decrease in MABP induced by 1.0 mg/kg of carvedilol. These observations strongly suggested that the low dose of carvedilol might increase CGRP release, thereby decreasing MABP in SHR. Since MABP was slightly, but siginificantly, increased with decreases in plasma levels of CGRP after administration of capsazapine 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 (23. Kawasaki et al., 1990).

Although both prazosin, a selective α_1-adrenoceptor antagonist, and bisoprolol, a selective β_1-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 β_2-adrenoceptor antagonist at doses of 0.25 and 1.0 mg/kg decreased MABP in SHR, while

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pretreatment with capsazepine only antagonized the decrease in MABP in animals given 0.25 mg/kg of ICI 118,551. ICI 118,551 at a dose of 0.25 mg/kg increased plasma levels of CGRP, while 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 β_2-adrenoceptor blockade activity. These observations are consistent with the previous reports demonstrating that β_2-adrenoceptor activation inhibited CGRP release from sensory neurons in *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 of carvedilol, while they were not increased by administration of 1.0 mg/kg of 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 of carvedilol were completely antagonized by pretreatment with capsazepine, suggesting that the low dose of carvedilol might increase 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 118551, 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 of ICI 118,551 were completely antagonized by pretreatment with capsazepine. Administration of capsaicin produced effects similar to those induced by low doses of carvedilol and ICI 118,551. 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 β_2-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 (Amuchasteguiet al., 1994), supporting the assumption described above. However, the reason why inhibition of β_2-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). Since 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 by inhibition of calcium influx into sensory neurons.

Treatment of CHF with β-adrenoceptor antagonists is considered advantageous, since 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 et al. (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 CSSN 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 β_2-adrenoceptor activation.

Although additional activities of carvedilol such as α-adrenoceptor blockade activity (van Zweiten, 1993), antioxidant activity (Feuerstein, et al., 1993), and anti-endothelin 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. (Cheng et al., 1999) demonstrated that carvedilol blocked K^+ and Ca^{2+} currents concomitantly in rabbit ventricular myocytes, suggesting that carvedilol might be beneficial in the treatment of ventricular tachyarrythmias 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 pathologic conditions of CHF by improving the hemodynamic conditions of CHF. Since this effect of carvedilol might depend on its β_2-adrenodceptor blockade activity as shown in the present study, the difference in the therapeutic effects in CHF between carvedilol and metoprolol, a selective β_1-adrenoceptor antagonist (Pole-Wilson et al., 2003), might be at least partly explained by the β_2-adrenoceptor blockade activity of carvedilol.

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Footnote.

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Figure legends

Fig. 1. Effects of carvedilol and/or capsazepine on mean arterial blood pressure in SHR.

Mean arterial blood pressure was continuously measured by pressure transducer after intravenous administration of 0.3 mg/kg (A) or 1.0 mg/kg (B) of carvedilol in SHR. Capsazepine (CPZ) (15 mg/kg) was injected subcutaneously 30 min prior to the administration of carvedilol. Control animals received saline instead of capsazepine or carvedilol. Each value represents the mean ± SD derived from 5 animal experiments. (A) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + carvedilol (0.3 mg/kg, iv); closed squares, CPZ + carvedilol (0.3 mg/kg, iv) : (B) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + carvedilol (1.0 mg/kg, iv); closed squares, CPZ + carvedilol (1.0 mg/kg, iv) . **, $p<0.01$ vs. saline; ††, $p<0.01$ vs. carvedilol.

Fig. 2. Effects of carvedilol and/or capsazepine on plasma levels of CGRP in SHR.

Plasma levels of CGRP in SHR were determined at 15 min after intravenous injection of 0.3 mg/kg or 1.0 mg/kg of carvedilol. Capsazepine (CPZ) (15 mg/kg) was subcutaneously injected 30 min prior to the administration of carvedilol. Control animals received saline instead of capsazepine or carvedilol. Each value represents mean ± SD derived from 6 animal experiments. **, $p<0.01$ vs. saline; ††, $p<0.01$ vs. carvedilol (0.3 mg/kg, iv); §§, $p<0.01$ vs. carvedilol (1.0 mg/kg, iv).

Fig. 3. Effects of prazosin, bisoprolol, ICI 118,551, capsaicin and/or capsazepine on mean arterial blood pressure in SHR.

Mean arterial blood pressure was continuously measured by pressure transducer after intravenous administration of prazosin (A), bisoprolol (B), 0.25 mg/kg of ICI 118,551 (C), 1.0 mg/kg of ICI 118,551 (D), and capsaicin (E) in SHR. Capsazepine (CPZ) (15 mg/kg) was injected subcutaneously (sc) 30 min prior to the administration of these agents. Control animals received saline instead of capsazepine, prazosin, or β-adrenoceptor antagonists and capsaicin. Each value represents the mean ± SD derived from 5 animal experiments. (A) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + prazosin (0.3 mg/kg, iv): closed squares, CPZ + prazosin (0.3 mg/kg, iv). (B) open circles, saline + saline; closed circle, CPZ + saline; open squares, saline + bisoprolol (0.3 mg/kg, iv); closed squares, CPZ + bisoprolol (0.3 mg/kg, iv). (C) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + ICI 118,551 (0.25 mg/kg, iv); closed squares, CPZ + ICI 118,551 (0.25 mg/kg, iv). (D) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + ICI 118,551 (1.0 mg/kg, iv); closed squares, CPZ + ICI 118,551 (1.0 mg/kg, iv). (E) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + capsaicin (1.0 mg/kg, sc): closed squares, CPZ + capsaicin (1.0 mg/kg, sc). **, $p<0.01$ vs. saline; ††, $p<0.01$ vs. ICI 118,551 (C) or capsaicin (E).

Fig. 4 Effects of prazosin, bisoprolol, ICI 118,551, capsaicin and/or capsazepine on plasma levels of CGRP in SHR.

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Plasma levels of CGRP in SHR were determined at 15 min after intravenous injection of prazosin (0.3 mg/kg), bisoprolol (0.3 mg/kg), and 0.3 mg/kg or 1 mg/kg of ICI 118,551 (ICI), 30 min after subcutaneous injection of capsaicin (1 mg/kg) and/or capsazepine. Capsazepine (CPZ) (15 mg/kg) was injected subcutaneously 30 min prior to the administration of these agents**.** Control animals received saline instead of capsazepine, prazosin, or β-adrenoceptor antagonists and capsaicin. Each value represents mean ± SD derived from 6 animal experiments. **, $p<0.01$ vs. saline; ††, $p<0.01$ vs. ICI (0.25 mg/kg, iv); §§, $p<0.01$ vs. ICI (1.0 mg/kg, iv); ¶¶, $p<0.01$ vs. capsaicin.

Fig. 5. Effects of carvedilol and/or capsazepine on renal tissue blood flow in SHR.

Renal tissue blood flow was continuously measured by laser-Doppler flowmeter after intravenous administration of 0.3 mg/kg of carvedilol (A), 1.0 mg/kg of carvedilol (B) and/or capsazepine in SHR. Capsazepine (CPZ) (15 mg/kg) was injected subcutaneously 30 min prior to the administration of carvedilol. Control animals received saline instead of capsazepine or carvedilol. Each value represents the mean ± SD derived from 5 animal experiments. (A) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + carvedilol (0.3 mg/kg, iv); closed squares, CPZ + carvedilol (0.3 mg/kg, iv). (B) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + carvedilol (1.0 mg/kg, iv); closed squares, CPZ + carvedilol (1.0 mg/kg, iv). **, $p<0.01$ vs. saline; ††, $p<0.01$ vs. carvedilol.

Fig. 6. Effecs of carvedilol and/or capsazepine on renal tissue levels of CGRP in SHR.

Renal tissue levels of CGRP in SHR were determined at 30 min after intravenous injection of 0.3 and 1.0 mg/kg of carvedilol and/or capsazepine. Capsazepine (CPZ) (15 mg/kg) was injected subcutaneously 30 min prior to the administration of carvedilol. Control animals received saline instead of capsazepine or carvedilol. Each value represents mean ± SD derived from 6 animal experiments. **, $p<0.01$ vs. saline; ††, $p<0.01$ vs. carvedilol (0.3 mg/kg, iv) ; §§, $p<0.01$ vs. carvedilol (1.0 mg/kg, iv).

Fig. 7. Effects of prazosin, bisoprolol, ICI 118,551, capsaicin and/or capsazepine on renal tissue blood flow in SHR.

Renal tissue blood flow was continuously measured by laser-Doppler flowmeter after intravenous administration of 0.25 mg/kg of ICI 118,551 (A), 1.0 mg/kg of ICI 118,551 (B), and capsaicin (C) and/or capsazepine in SHR. Capsazepine (CPZ) (15 mg/kg) was injected subcutaneously (sc) 30 min prior to the administration of these agents. Control animals received saline instead of capsazepine, prazosin, or β-adrenoceptor antagonists and capsaicin. Each value represents the mean ± SD derived from 5 animal experiments. (A) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + ICI 118,551 (0.25 mg/kg, iv); closed squares, CPZ + ICI 118,551 (0.25 mg/kg, iv). (B) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + ICI 118,551 (1.0 mg/kg, iv); closed squares, CPZ + ICI 118,551 (1.0 mg/kg, iv). (C) open circles, saline + saline; closed circles, CPZ + saline; open squares, saline + capsaicin (1.0 mg/kg, sc); closed squares, CPZ +

capsaicin (1.0 mg/kg, sc). **, p<0.01 vs. saline; ††, p<0.01 vs. ICI 118,551 (A, B) or capsaicin (C).

Fig. 8. Effects of prazosin, bisoprolol, ICI 118,551, capsaicin and/or capsazepine on renal tissue levels of CGRP in SHR.

Renal tissue levels of CGRP in SHR were determined at 30 min after intravenous injection of 0.3 and 1.0 mg/kg of ICI 118,551 (ICI), or 45 min after subcutaneous (sc) injection of capsaicin (1.0 mg/kg) and/or capsazepine. Capsazepine (CPZ) (15 mg/kg) was injected subcutaneously 30 min prior to the administration of ICI 118,551 and capsaicin. Control animals received saline instead of capsazepine, prazosin, or β-adrenoceptor antagonists and capsaicin. Each value represents mean ± SD derived from 6 animal experiments. **, p<0.01 vs. saline; ††, p<0.01 vs. ICI (0.25 mg/kg, iv); §§, p<0.01 vs. ICI (1.0 mg/kg, iv); ¶¶, p<0.01 vs. capsaicin.

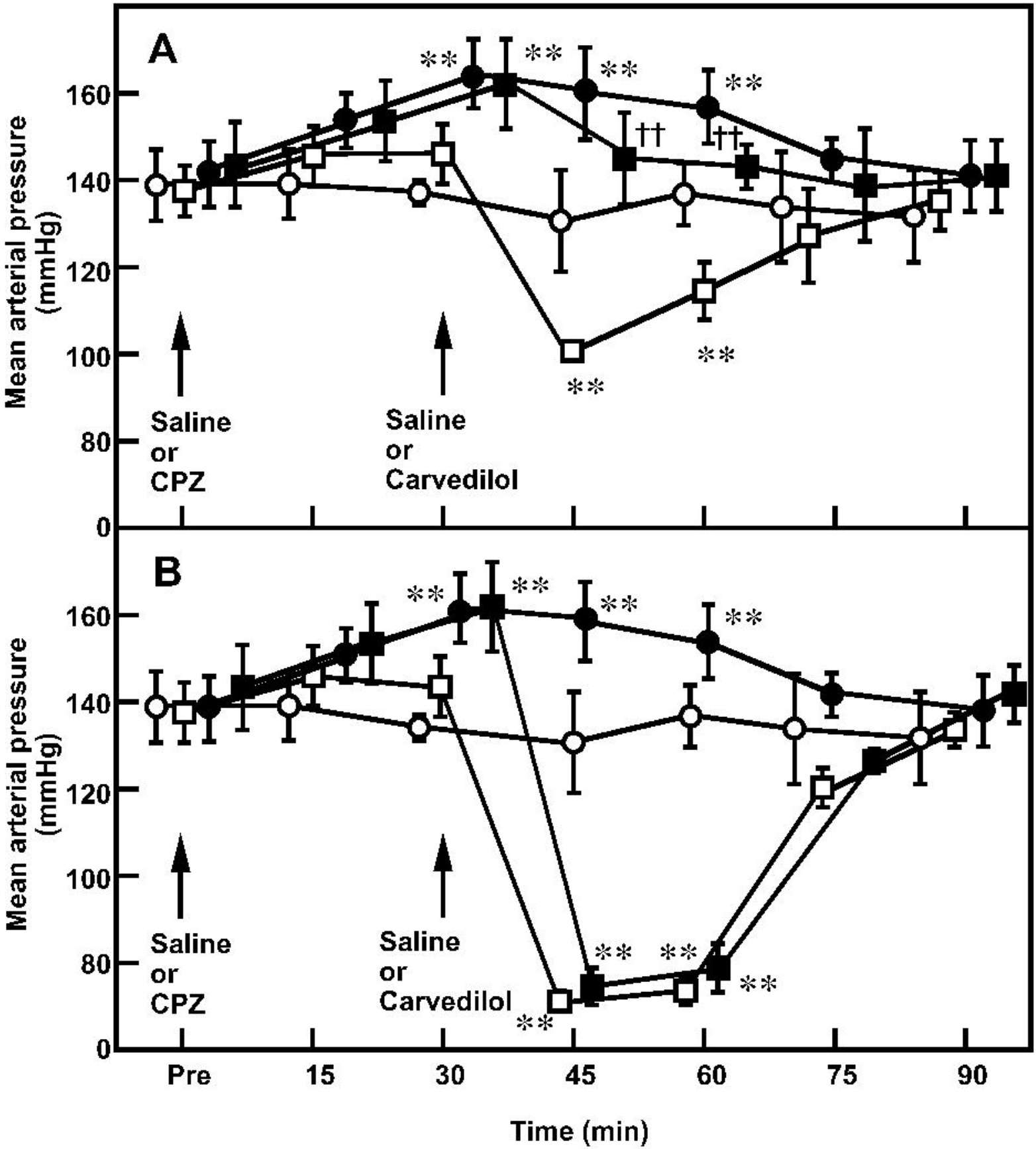
A
B
Mean arterial pressure (mmHg)
160
140
120
100
80
0
Saline or CPZ
Saline or Carvedilol
**
††
Pre
15
30
45
60
75
90
Time (min)

Figure 1

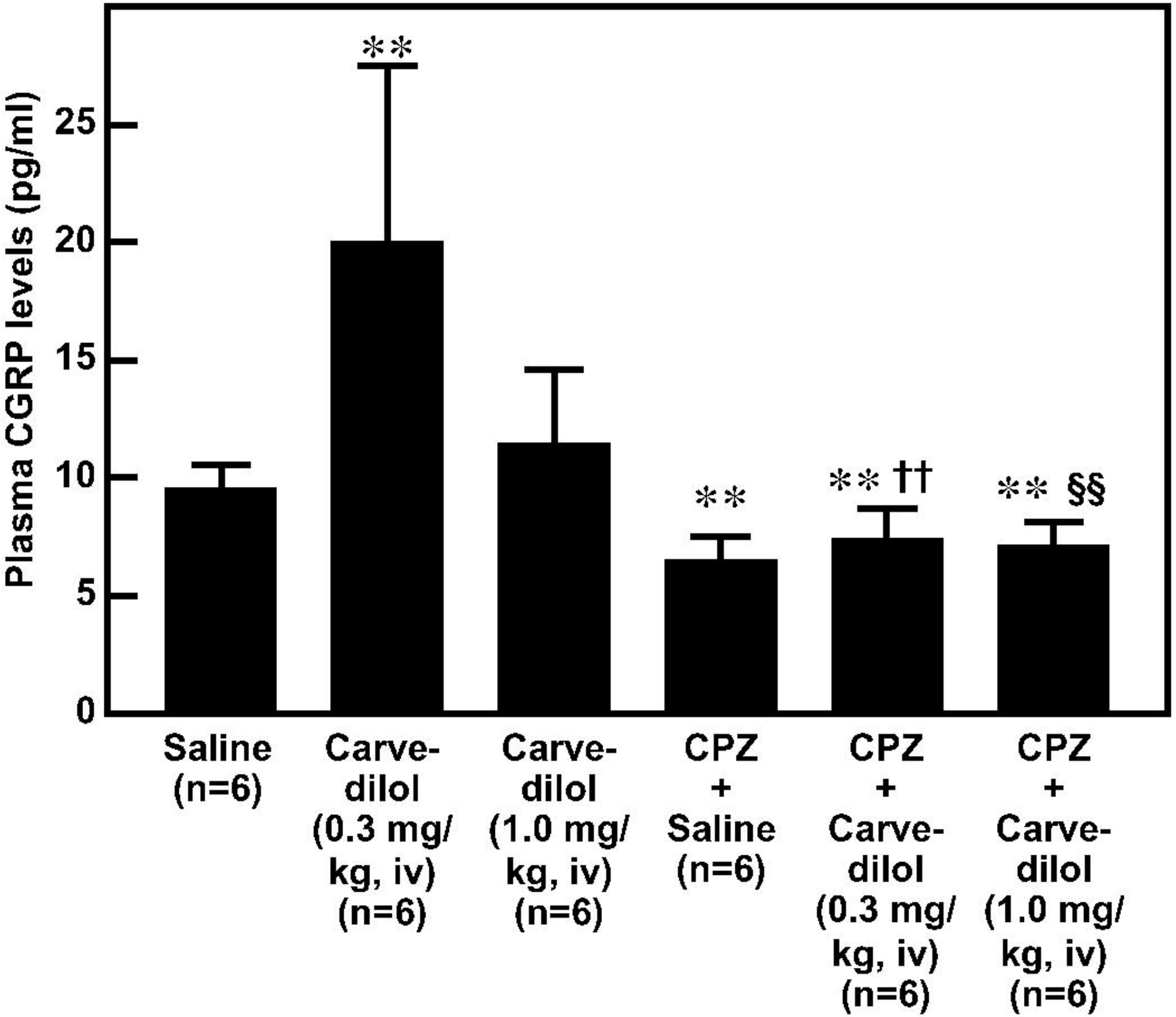
Plasma CGRP levels (pg/ml)
0
5
10
15
20
25
**
**
** ††
** §§
Saline (n=6)
Carve-dilol (0.3 mg/ kg, iv) (n=6)
Carve-dilol (1.0 mg/ kg, iv) (n=6)
CPZ + Saline (n=6)
CPZ + Carve-dilol (0.3 mg/ kg, iv) (n=6)
CPZ + Carve-dilol (1.0 mg/ kg, iv) (n=6)

Figure 2

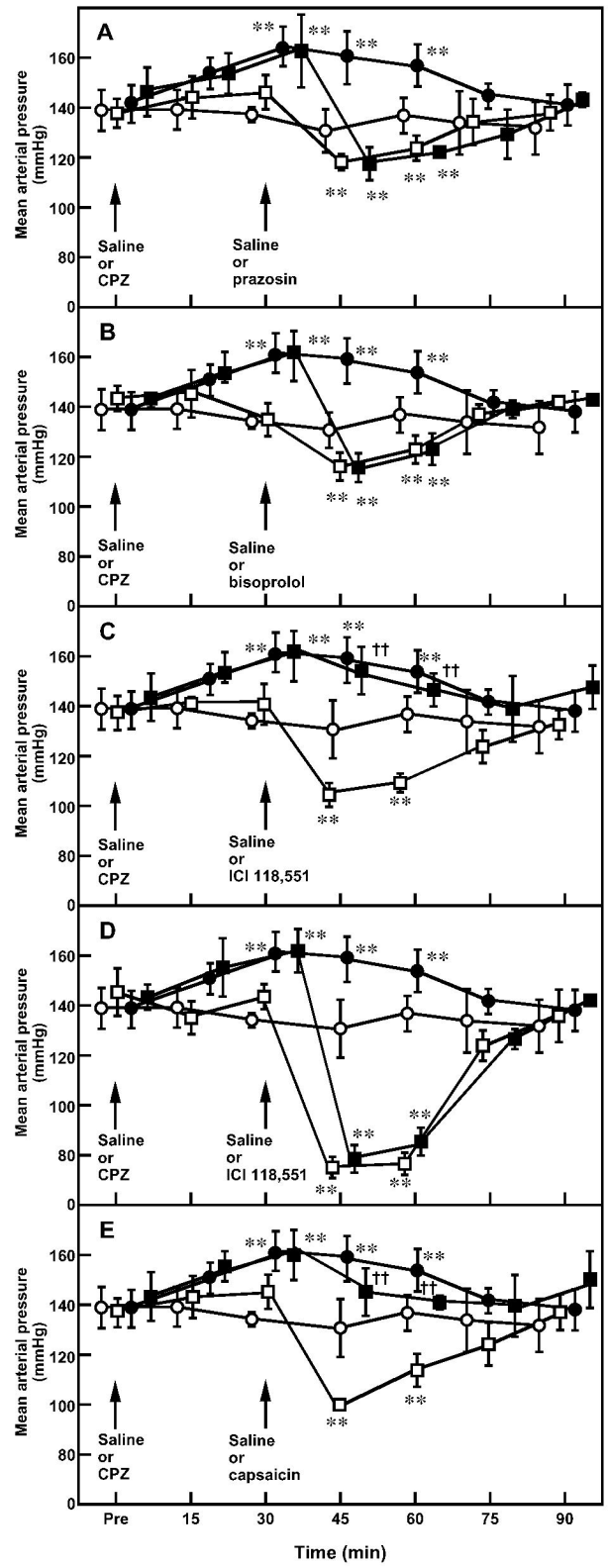
A
B
C
D
E
Mean arterial pressure (mmHg)
160
140
120
100
80
0
Saline or CPZ
Saline or prazosin
Saline or bisoprolol
Saline or ICI 118,551
Saline or ICI 118,551
Saline or capsaicin
**
††
Pre
15
30
45
60
75
90
Time (min)

Figure 3

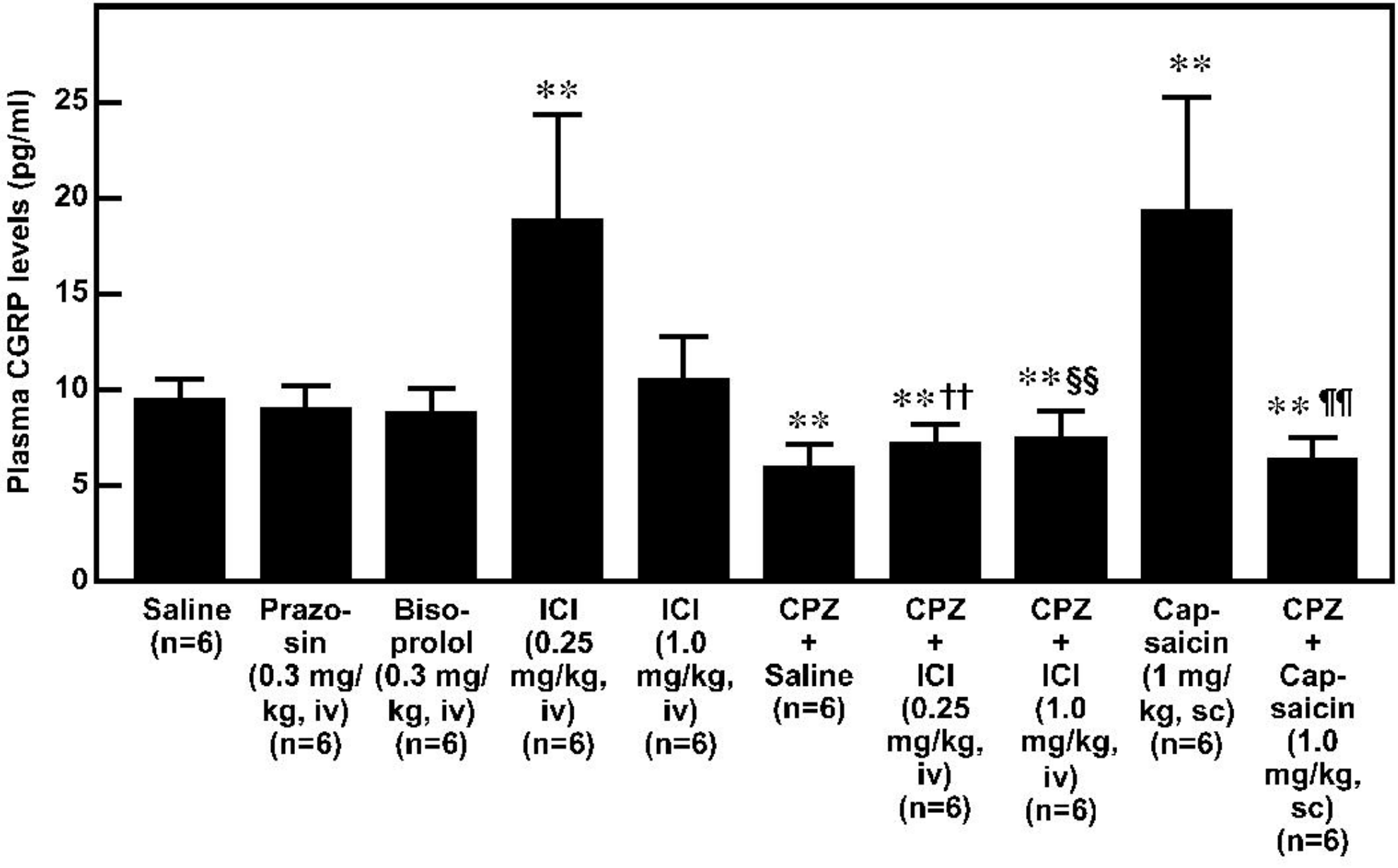
Plasma CGRP levels (pg/ml)
0
5
10
15
20
25
**
**
**††
**§§
**
**¶¶
Saline (n=6)
Prazo-sin (0.3 mg/ kg, iv) (n=6)
Biso-prolol (0.3 mg/ kg, iv) (n=6)
ICI (0.25 mg/kg, iv) (n=6)
ICI (1.0 mg/kg, iv) (n=6)
CPZ + Saline (n=6)
CPZ + ICI (0.25 mg/kg, iv) (n=6)
CPZ + ICI (1.0 mg/kg, iv) (n=6)
Cap-saicin (1 mg/ kg, sc) (n=6)
CPZ + Cap-saicin (1.0 mg/kg, sc) (n=6)

Figure 4

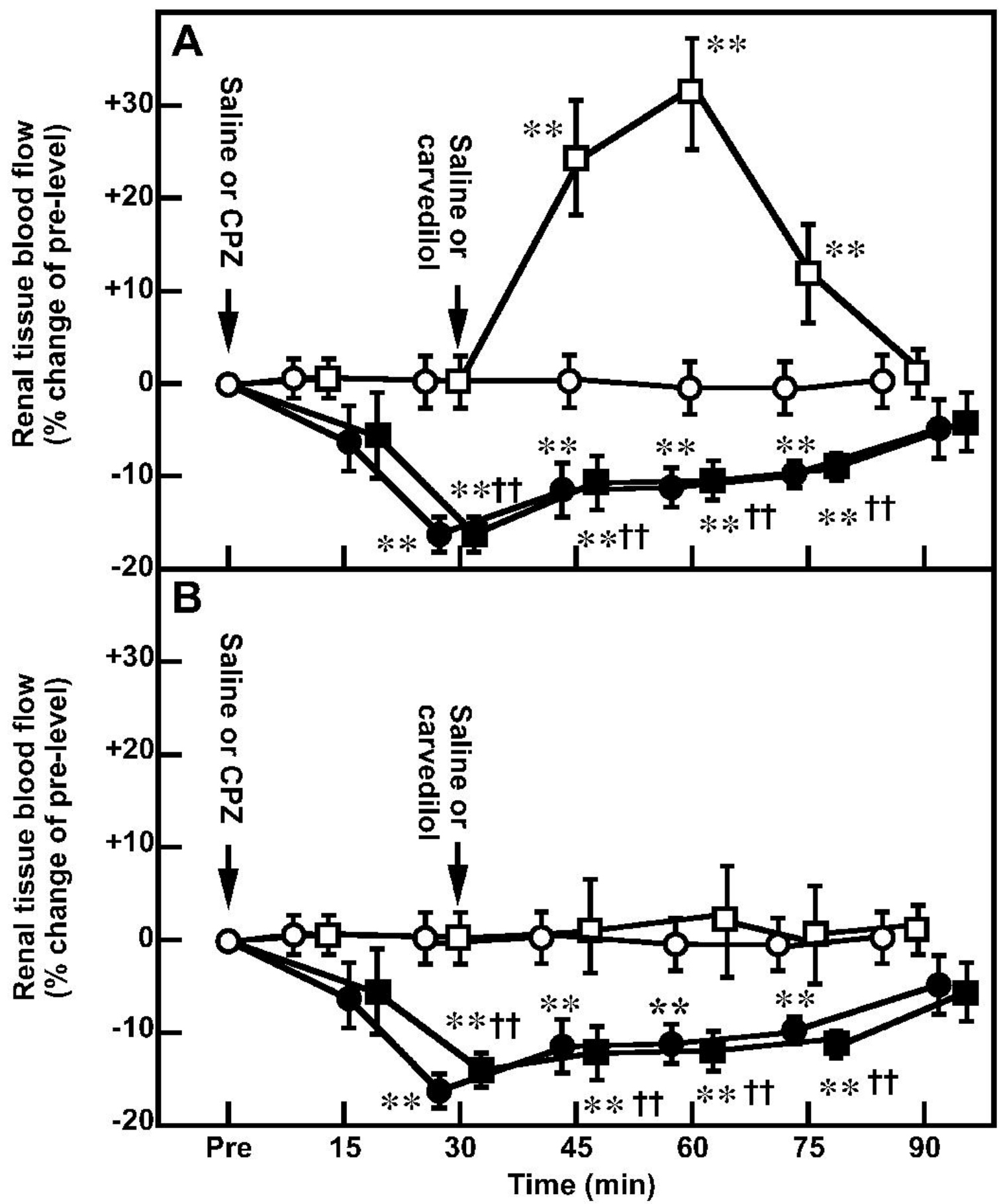
A
B
Saline or CPZ
Saline or carvedilol
Renal tissue blood flow (% change of pre-level)
+30
+20
+10
0
-10
-20
**
**††
††
Pre
15
30
45
60
75
90
Time (min)

Figure 5

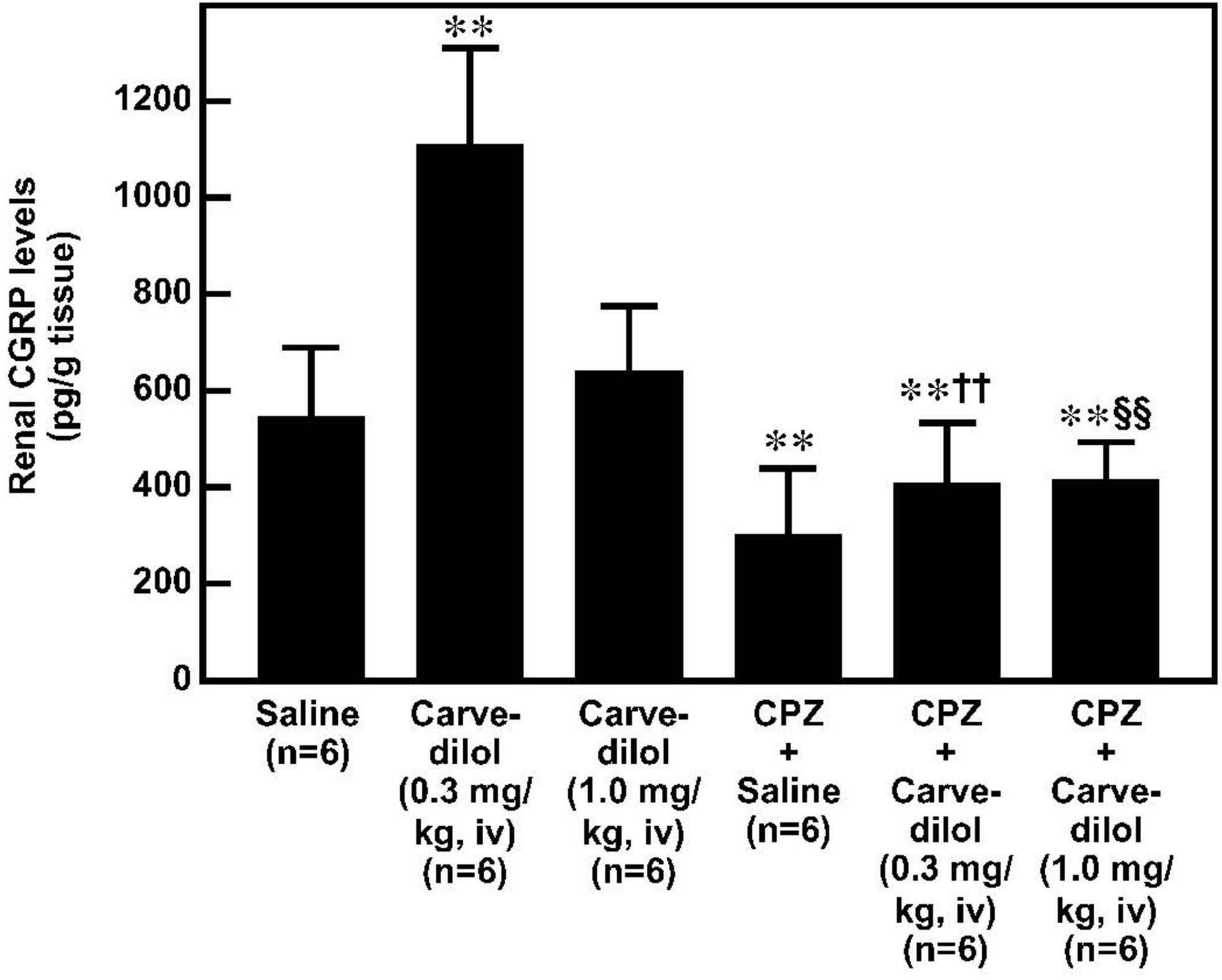
Renal CGRP levels (pg/g tissue)
0
200
400
600
800
1000
1200
**
**
**††
**§§
Saline (n=6)
Carve-dilol (0.3 mg/ kg, iv) (n=6)
Carve-dilol (1.0 mg/ kg, iv) (n=6)
CPZ + Saline (n=6)
CPZ + Carve-dilol (0.3 mg/ kg, iv) (n=6)
CPZ + Carve-dilol (1.0 mg/ kg, iv) (n=6)

Figure 6

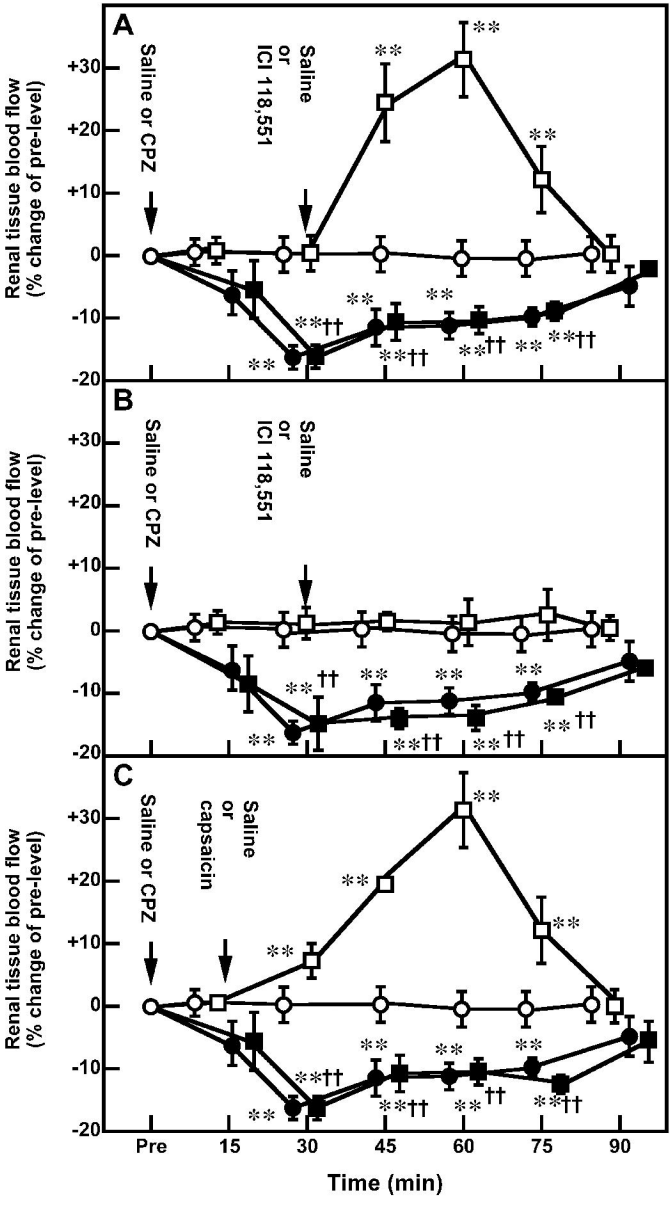
A
B
C
Saline or CPZ
Saline or ICI 118,551
Saline or capsaicin
Renal tissue blood flow (% change of pre-level)
+30
+20
+10
0
-10
-20
Pre
15
30
45
60
75
90
Time (min)

Figure 7

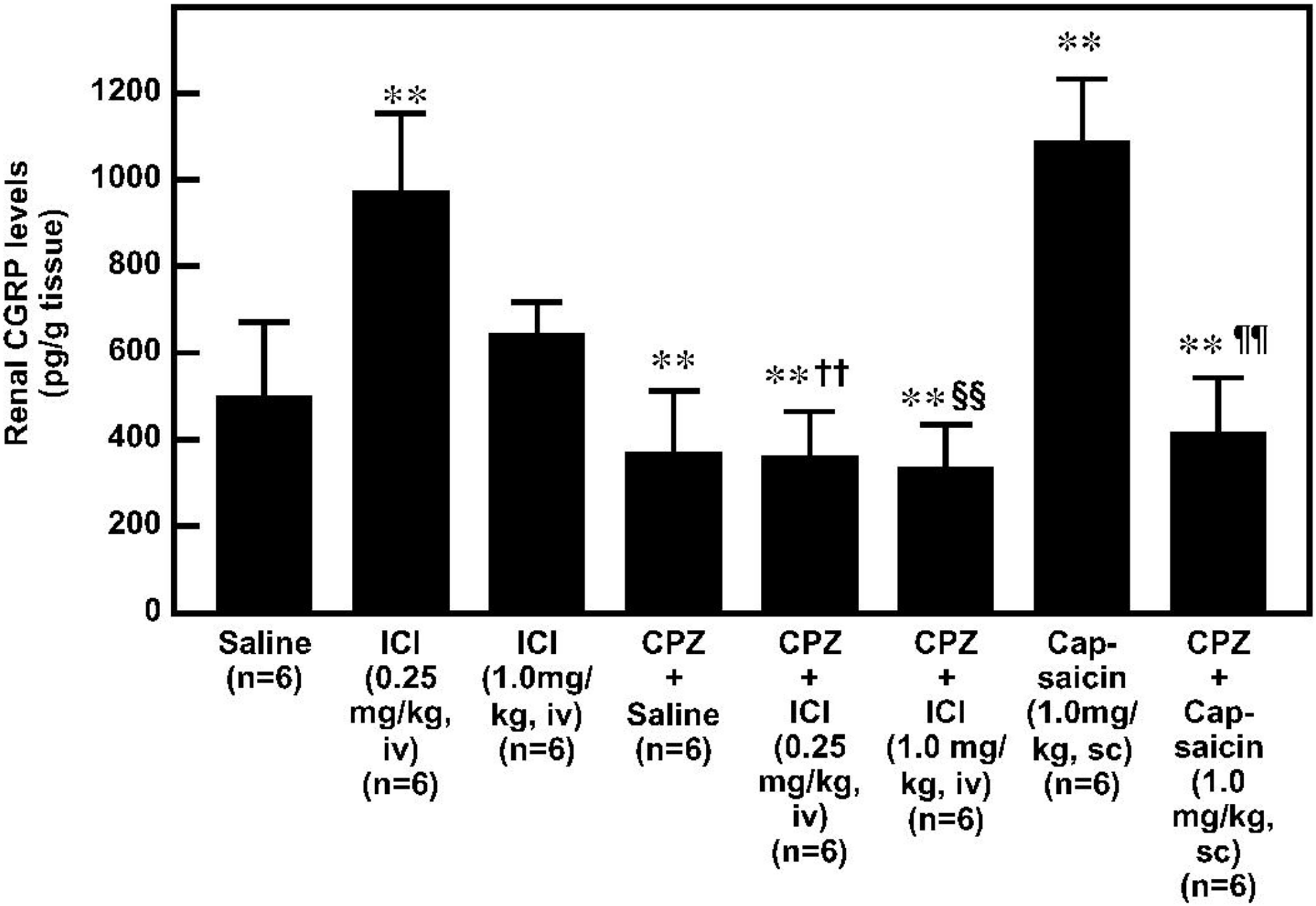
Renal CGRP levels (pg/g tissue)
0
200
400
600
800
1000
1200
**
**
**††
**§§
**
** ¶¶
Saline (n=6)
ICI (0.25 mg/kg, iv) (n=6)
ICI (1.0mg/ kg, iv) (n=6)
CPZ + Saline (n=6)
CPZ + ICI (0.25 mg/kg, iv) (n=6)
CPZ + ICI (1.0 mg/ kg, iv) (n=6)
Cap-saicin (1.0mg/ kg, sc) (n=6)
CPZ + Cap-saicin (1.0 mg/kg, sc) (n=6)

Figure 8