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Protection by capsaicin against attenuated endothelium-dependent vasorelaxation due to lysophosphatidylcholine

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Abstract Previous studies have shown that pretreatment with calcitonin gene-related peptide (CGRP), a principal transmitter in sensory nerves, can protect the endothelial cell. We therefore evaluated whether in vivo capsaicin treatment prevents endothelial damage elicited by lysophosphatidylcholine (LPC) in the rat aorta. Acute treatment or repeated pretreatment with capsaicin resulted in stimulation of neurotransmitter release from sensory nerves or depletion of their transmitter content respectively. Vasodilator responses to acetylcholine (ACh) were examined in the aorta of these animals. Acute application of capsaicin (50 mg/kg) increased the plasma concentration of CGRP-like immunoreactivity (CGRP-LI) concomitantly with a reversal of the inhibition by LPC of endothelium-dependent ACh-induced relaxation in the isolated rat aorta. After repeated pretreatment with capsaicin to deplete sensory nerve neurotransmitter content the effects of capsaicin were absent as shown by the plasma CGRP-LI concentration and the vasodilator response to ACh. The results demonstrate that systemic capsaicin treatment, which evokes the release of CGRP from sensory nerves, protects the endothelial cell. The present study also suggests that CGRP may be an endogenous vascular protective substance.

Key words Capsaicin · CGRP (calcitonin gene-related peptide) · Endothelium-dependent relaxation · Thoracic aorta · Rat

Introduction

Capsaicin selectively stimulates the release of neurotransmitters from sensory nerves and is used as a tool to study the physiological and pharmacological properties of capsaicin-sensitive sensory nerves (Buck and Burks 1986). Previous studies have suggested that capsaicin affords protection of the rat gastric mucosa against ethanol-induced damage (Holzer and Lippe 1988). Recently, studies have shown that capsaicin reduces myocardial injury due to ischaemia-reperfusion in the isolated perfused rat heart (D'Alonzo et al. 1995; Li et al. 1996).

Calcitonin gene-related peptide (CGRP), a principal neurotransmitter in sensory nerves, is distributed widely in vascular tissues in both the central nervous system and the periphery (Gibson et al. 1984; Wharton et al. 1986). CGRP, besides relaxing vascular smooth muscle, has a protective effect on the myocardium and the endothelial cell (Lopez-Belmonte et al. 1993). In the isolated perfused rat heart, pretreatment with CGRP protects against myocardial injury induced by ischaemia-reperfusion (Li et al. 1996) and in the rabbit thoracic aorta and vascular endothelial cells cultured from bovine aorta, CGRP also prevents endothelial cell damage due to oxidized low-density lipoprotein (Li et al. 1995). Oxidized low-density lipoprotein and its major component lysophosphatidylcholine (LPC) have been shown to play an important role in endothelial damage in cardiovascular diseases such as atherosclerosis (Parthasarathy et al. 1989; Kugiyama et al. 1990) and studies in vitro have shown that LPC attenuates endothelium-dependent relaxation (Li et al. 1995).

In view of the stimulatory effect of capsaicin on CGRP release and the protective effect of exogenous CGRP on the endothelial cell, we evaluated in the present study the effect of systemic capsaicin treatment on endothelial cell damage elicited by LPC in the isolated rat thoracic aorta.

Materials and methods

Capsaicin treatment in vivo. Male Wistar rats weighing 180–250 g were used. Animals were treated with capsaicin (dissolved in a vehicle containing 10% Tween 80, 10% ethanol and 80% saline) by s.c. injection under ether anaesthesia. For the study on capsaicin-induced acute release of CGRP from sensory nerves, rats were treated with a single dose of capsaicin (50 mg/kg). Preliminary experiments showed that 1 h after pretreatment with capsaicin the plasma concentration of CGRP-like immunoreactivity (CGRP-LI)

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was slightly increased (71.2 ± 6.4 ng/l, n = 3). Others have reported that blood flow in the rat stomach is increased significantly 90–150 min after capsaicin administration (Holzer et al. 1990). Thus, in the present study measurements of the CGRP content and endothelium functions were made 3 h after treatment with capsaicin. For repeated administration of capsaicin, rats received 50 mg/kg capsaicin followed by a second injection of 50 mg/kg 24 h later, as described previously (Virus et al. 1983). To confirm the depletion of transmitter content in sensory nerves and to rule out a direct effect of capsaicin on endothelial cells, after repeated capsaicin administration for 4 days, the animals were again injected with capsaicin (50 mg/kg) 3 h prior to the experiments. Control rats were injected with vehicle alone.

Determination of plasma CGRP-LI concentration. After acute administration of capsaicin, the rats were anaesthetized by an i.p. injection of pentobarbitone sodium (60 mg/kg). Blood samples (3 ml) were collected from the carotic artery in tubes containing 10% Na_2EDTA 30 µl and aprotinin 400 mU/l. Serum was obtained by centrifugation at 1300 × g for 20 min (4° C). CGRP-LI in plasma was determined by radioimmunoassay kits using antisera raised against rat CGRP, ^{125}I-labelled CGRP (specific activity 1415 Ci/mol) and rat CGRP standard.

Organ bath experiments. The thoracic aorta was rapidly isolated and cut into rings of 4 mm length. These were then suspended horizontally between two stainless steel wires and mounted in a 5-ml organ chamber filled with warmed (37° C) and oxygenated (95% O_2 and 5% CO_2) Krebs' solution. The Krebs' solution had the following composition (mM): NaCl, 119.0; $NaHCO_3$, 25.5; KCl, 4.3; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; EDTA, 0.026 and glucose, 11.0. One end of the ring was connected to a force transducer and a resting tension of 2 g applied. The rings were equilibrated for 60 min and then precontracted with KCl (40 mM). After a maximal response to KCl was obtained, the rings were washed repeatedly with Krebs' solution and equilibrated again for 30 min before measuring vasodilator responses to acetylcholine (ACh).

Five groups of rats were studied: control, LPC, acute application of capsaicin in vivo, repeated administration of capsaicin in vivo, and treatment with capsaicin in vitro. To measure vasodilator responses, the aortic rings were precontracted with phenylephrine to 40–50% of their maximal contraction. After the contractions stabilized, a cumulative concentration response curve to ACh (10^{-9} – 10^{-6} M) was obtained. In the control group, measurements of vasodilator responses to ACh were made. For LPC, rings were exposed to LPC (5 µg/ml) for 30 min before addition of phenylephrine to increase vascular tone, and then measurements of vasodilator responses to ACh were made in the presence of LPC. For systemic capsaicin treatment, the rings were exposed to LPC for 30 min before addition of phenylephrine to increase vascular tone, and then measurements of vasodilator responses to ACh were made in the presence of LPC. For the study on the direct effect of capsaicin on vasodilator responses to ACh, the rings were exposed to capsaicin (3×10^{-7} M) for 30 min before addition of phenylephrine to increase vascular tone, and then measurements of vasodilator responses to ACh were made.

Reagents. LPC, phenylephrine, ACh, CGRP and capsaicin were obtained from Sigma (St Louis, Mo., USA). Radioimmunoassay kits for measurement of CGRP were obtained from Dongya Immunity Technology Institution (P.R. China).

Statistical analysis. All data were analysed using a two-way ANOVA. All values were expressed as means ± SEM. The significance level was chosen as $P < 0.05$.

Results

Plasma concentration of CGRP-LI

As shown in Fig. 1, the plasma CGRP-LI concentrations in the rats treated with a single dose of capsaicin were significantly increased compared with those of control rats. However, the level of CGRP-LI was no longer increased after repeated administration of capsaicin.

Effect of capsaicin on vasodilator responses to acetylcholine (ACh)

Phenylephrine was added to increase smooth muscle tone in the aortic rings. The active tension generated was 1.02 ± 0.06 g, 1.16 ± 0.07 g and 1.16 ± 0.07 g for control, acute treatment and repeated pretreatment with capsaicin, respectively (n = 6). Exposure of the aortic ring to capsaicin (3×10^{-7} M) did not affect vasoconstrictor responses to phenylephrine (the tension was 1.03 ± 0.08 g, n = 6). Incubation with LPC (5 µg/ml) evoked a slight increase in constrictor responses to phenylephrine (the tension was 1.1 ± 0.1 g, n = 6), but there were no significant differences compared with the untreated preparation ($P > 0.05$).

In the presence of phenylephrine (10^{-7} M), ACh caused concentration-dependent relaxation of the isolated rat aorta. Vasodilator responses to ACh at concentrations of 10^{-8}–10^{-6} M were significantly reduced in the presence of LPC (5 µg/ml). After acute application of capsaicin, the inhibition by LPC of vasodilator responses to ACh at concentrations of 10^{-8}–10^{-6} M was significantly attenuated. However, after repeated pretreatment with capsaicin, the effect of capsaicin was abolished, as shown by the reappearance of inhibition by LPC of the vasodilator responses to ACh (Fig. 2).

To rule out a direct effect of capsaicin on the endothelium, the influence of capsaicin on vasodilator responses to ACh in the isolated thoracic aorta was tested. Incuba-

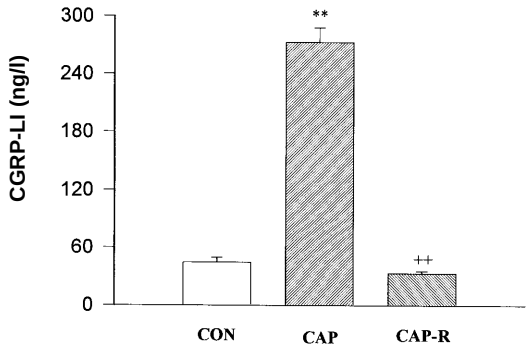

Fig. 1 The plasma concentration of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) after acute or repeated application of capsaicin. *CON*: treatment with vehicle; *CAP*: treatment with a single dose of capsaicin; *CAP-R*: repeated treatment with capsaicin. Means ± SEM, n = 6. ** $P < 0.01$ vs. CON; $^{++}P < 0.01$ vs. CAP

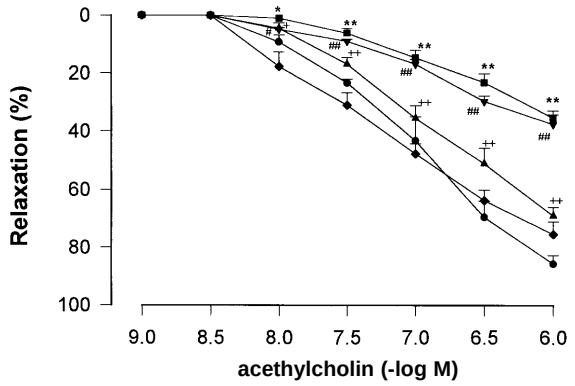

Fig. 2 Effect of capsaicin on the inhibition by lysophosphatidylcholine (LPC) of endothelium-dependent vasorelaxation to acetylcholine in the isolated rat thoracic aorta. Tissues were precontracted with phenylephrine (10^{-7}–10^{-6} *M*). Symbols: ● control; ◆ treatment with capsaicin (3×10^{-7} *M*) in vitro; ■ LPC, 5 μg.ml^{-1}; ▲ LPC after acute application of capsaicin (50 mg/kg) in vivo; ▼ LPC after repeated administration of capsaicin in vivo. Means ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; $^{+}P < 0.05$, $^{++}P < 0.01$ vs. LPC, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. acute application of capsaicin

tion with capsaicin alone (3×10^{-7} *M*) had no effect on vasodilator responses to ACh (Fig. 2).

Discussion

The present study shows that acute application of capsaicin reverses the attenuation of the ACh-induced endothelium-dependent relaxation by LPC in the rat thoracic aorta, while the protective effect of capsaicin is absent in the rats pretreated repeatedly with capsaicin. Systemic capsaicin desensitization has been reported to enhance experimentally induced ulceration in the rat gastric mucosa, and intragastric capsaicin affords protection of the rat gastric mucosa against ethanol-induced damage (Holzer and Lippe 1988). More recently, pretreatment with capsaicin has been shown to protect against myocardial injury induced by ischemia-reperfusion in the isolated perfused rat heart (D'Alonzo et al. 1995; Li et al. 1996). These results suggest that sensory nerves may be involved in protective effects in multiple tissues.

Capsaicin has been shown to have complex pharmacological actions. As mentioned above, capsaicin has a protective effect on the endothelium in the microcirculation of rat stomach (Holzer et al. 1990). In contrast, capsaicin causes an increase in vascular permeability in the microcirculation of rat skin (Kenins et al. 1984). To rule out the direct effect of capsaicin on the endothelium, we examined the influence of capsaicin on vasodilator responses to ACh in the isolated thoracic aorta. In the present study capsaicin itself did not affect vasodilator responses to ACh. The discrepancy with our results may be due to the different tissues studied. In addition, as discussed in more detail below, capsaicin stimulates release of multiple peptides which have different pharmacological properties. Substance P, which co-exists with CGRP in sensory nerves, exerts a vasodilator response and an increase in the vascular permeability in the skin microcirculation (Lopez-Belmonte and Whittle 1993), whereas CGRP has a protective effect on the endothelium in the microcirculation of the rat skin (Kjartansson et al. 1987). These studies also suggest that neuropeptides from sensory nerves have different pharmacological properties.

Capsaicin-sensitive sensory nerves contain a number of peptides, including CGRP, substance P and neurokinin A (Franco-Cereceda 1988). Besides CGRP, substance P and neurokinin A could also mediate the effect of capsaicin. Among these peptides CGRP has been shown to possess a beneficial effect of the myocardium and the endothelial cell. We and others have shown that in the isolated rat heart, pretreatment with capsaicin protects against myocardial injury induced by ischaemia-reperfusion, an effect which is due to stimulation of CGRP release in cardiac sensory nerves (D'Alonzo et al. 1995; Li et al. 1996). Pretreatment with CGRP also reduces myocardial injury due to ischaemia-reperfusion (Li et al. 1996). Recently we showed that exogenous CGRP protects endothelial cells in the rabbit thoracic aorta and vascular endothelial cells cultured from bovine aorta against damage due to oxidized low-density lipoprotein (Li et al. 1995). In the present study, acute capsaicin treatment of rats caused an increase in the plasma concentration of CGRP-LI concomitantly with an attenuation of the inhibition by LPC of vasodilator responses to ACh in the thoracic aorta, while after repeated pretreatment with capsaicin the plasma level of CGRP-LI was decreased and the protective effect of capsaicin was absent. These studies suggest that CGRP may be involved in the mediation of the protective effect of capsaicin on the endothelium. Although there is evidence suggesting that substance P or neurokinin A released from peripheral nerve endings of afferent nerve fibre causes only vasodilation, an increase in the vascular permeability to macromolecules and contraction of smooth muscles in bronchi (Pernow 1983; Maggio 1988), we can not exclude the possibility that either substance P or neurokinin A is beneficial to the endothelial cell, although direct protection of either has not yet been reported.

We have shown recently that in the isolated perfused rat heart, CGRP receptor antagonists abolished the cardioprotection of ischaemic preconditioning (Xiao et al. 1996), and that pretreatment with CGRP or capsaicin can substitute for preconditioning ischaemia (Li et al. 1996). These studies suggest that CGRP may be an endogenous myocardial protective substance. There is evidence to suggest that the actions of sensory nerves (CGRP) are decreased in the mesenteric arterial bed of the diabetic or hypertensive rat, and endothelial cells in these animals also exhibit reduced vasodilator responses (Kawasaki et al. 1990; Ralevic et al. 1993). In the present study, the protective effect of capsaicin was abolished in rats repeatedly pretreated with capsaicin to deplete neurotransmitter content in sensory nerves. Other investigators have also shown the protective effect of CGRP against LPC-induced microvascular injury (Lopez-Belmonte and Whittle 1993). These results, together with observations that pre-

treatment with CGRP protects against endothelial cell damages due to oxidized low-density lipoprotein in the rabbit thoracic aorta and vascular endothelial cells cultured from bovine aorta, suggest that CGRP may also be an endogenous vascular protective substance.

The mechanisms responsible for the protective effect of CGRP on endothelial cells are unclear. There is evidence to suggest that, in cultured endothelial cells, the protection of anoxic or pharmacological preconditioning is mediated via the protein kinase C (PKC) pathway (Zhou et al. 1996). In adult mammalian ventricular cardiomyocytes, CGRP increases the activation of PKC (Bell et al. 1995). More recently, we have shown that the protective effects of CGRP in the isolated rat heart or in the rabbit and rat aorta are abolished by an inhibitior of PKC (Peng et al. 1996; Tang et al. 1996). Thus we postulate that the protective effect of capsaicin on the endothelium may be related to the activation of the PKC pathway by the release of CGRP from sensory nerves. However, further work is needed to establish this hypothesis.

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