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Involvement of calcitonin gene-related peptide (CGRP) receptors in insulin-induced vasodilatation in mesenteric resistance blood vessels of rats

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1 The vascular effect of insulin in the mesenteric resistance blood vessel and the role of calcitonin gene-related peptide (CGRP)-receptor in insulin-induced vascular responsiveness were investigated in rats.

2 The mesenteric vascular beds isolated from Wistar rats were perfused with Krebs solution, and perfusion pressure was measured with a pressure transducer. In preparations contracted by perfusion with Krebs solution containing methoxamine in the presence of guanethidine, the perfusion of insulin (from 0.1 to 3000 nM) caused a concentration-dependent decrease in perfusion pressure due to vasodilatation. The pD_2 value and maximum relaxation (%) were 6.94 ± 0.22 and 43.9 ± 5.2, respectively.

3 This vasodilator response to insulin was unaffected by 100 nM propranolol (β-adrenoceptor antagonist) plus 100 nM atropine (muscarinic cholinoceptor antagonist), 100 μM L-N^G-nitroarginine (nitric oxide synthase inhibitor), 1 μM ouabain (Na^+-K^+ ATPase inhibitor), or 1 μM glibenclamide (ATP sensitive K^+-channel inhibitor).

4 In preparations without endothelium, perfusion of insulin produced a marked vasodilatation. The pD_2 value and maximum relaxation (%) were 7.62 ± 0.21 and 81.0 ± 4.6, respectively, significantly greater than in preparations with intact endothelium.

5 The vasodilator responses to insulin in the preparations without endothelium were significantly inhibited by CGRP[8–37], a CGRP receptor antagonist, whereas pretreatment with capsaisin, a toxin for CGRP-containing nerves, did not affect insulin-induced vasodilatation.

6 These results suggest that insulin induces non-adrenergic, non-cholinergic and endothelium-independent vasodilatation, which is partially mediated by CGRP receptors.

Keywords: Insulin; vasorelaxation; mesenteric resistance blood vessel; calcitonin gene-related peptide (CGRP); CGRP receptors

Introduction

Essential hypertension is frequently associated with insulin resistance and hyperinsulinaemia (Manicardi *et al*., 1986; Ferrannini *et al*., 1987; Pollare *et al*., 1990). This relationship suggests that insulin resistance and hyperinsulinaemia contribute to the pathogenesis of hypertension, and furthermore, that insulin might play an important role in pathophysiological regulation of the cardiovascular system. However, the effects of insulin on blood pressure are complex. Several investigators have shown that insulin activates the sympathetic noradrenergic system and increases vascular resistance and arterial pressure (Landsberg, 1986; Reaven, 1988). In contrast, recent studies have shown that insulin acts as an endogenous vasodilator. Vasodilator effects of insulin have been observed in laboratory animals (Liang *et al*., 1982; Gros *et al*., 1994), and in the human forearm, skeletal muscle, and lower limb arteries (Creager *et al*., 1985; Anderson *et al*., 1991; Steinberg *et al*., 1993). However, how insulin mediates vasodilatation in these arteries remains obscure. An insulin-mediated vascular response has been found in some isolated elastic arteries, but, whether resistance blood vessels respond to insulin is unknown.

Recently, calcitonin gene-related peptide (CGRP), which is a potent vasodilator (Brain *et al*., 1985; Kawasaki *et al*., 1988), has been shown to inhibit insulin-stimulated glucose uptake into skeletal muscle. Amyline, which is structurally related to CGRP and does not induce vasodilatation, also inhibits the effect of insulin. These findings raise the possibility that insulin may interact with CGRP receptors. Because CGRP is a potent vasodilator (Brain *et al*., 1985) and acts as a vasodilator neurotransmitter in non-adrenergic, non-cholinergic (NANC) nerves (Kawasaki *et al*., 1988), this peptide is considered to be involved in the control of the tone of resistance blood vessels (Kawasaki *et al*., 1988; 1990; Han *et al*., 1990). Thus, it is possible that the mechanism of insulin-induced vasodilatation involves NANC vasodilator nerves. However, little is known about the role of CGRP-containing vasodilator nerves in insulin-induced vascular responsiveness.

In this study, we investigated the mechanisms underlying insulin-induced vasodilatation in mesenteric resistance blood vessels and showed that CGRP-receptors are involved in insulin-induced vasorelaxation.

Methods

Perfusion of mesenteric vascular beds

Male Wistar rats at 8 weeks of age were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.) and their mesenteric vascular beds isolated and prepared for perfusion as described previously (Kawasaki *et al*., 1988). The superior mesenteric artery was cannulated and flushed gently with Krebs-Ringer

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bicarbonate solution (Krebs solution) in order to eliminate blood in the vascular bed. After removal of the entire intestine and associated vascular bed, the mesenteric vascular bed was separated from intestine by cutting close to the intestinal wall. Only four main arterial branches from the superior mesenteric trunk running to the terminal ileum were perfused. All other branches of the superior mesenteric artery were tied off. Isolated mesenteric vascular beds were then placed in a water jacketed organ bath maintained at 37°C and perfused with a modified (see below) Krebs solution at a constant flow rate of 5 ml min^{-1} with a peristaltic pump (SJ-1215, ATTO, Tokyo, Japan). Preparations were also superfused with the same solution at the rate of 0.5 ml min^{-1} to prevent drying. The Krebs solution was bubbled with a mixture of 95% O_2-5% CO_2 before passage through a warming coil maintained at 37°C. Modified Krebs solution was of the following composition (mM): NaCl 120.0, KCl 5.0, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, disodium EDTA 0.027 and dextrose 11.0 (pH 7.4). Changes in the perfusion pressure were measured with a pressure transducer (MPU-0.5A, Nihon Kohden, Tokyo, Japan) and recorded on a polygraph (RM-25, Nihon Kohden).

Perfusion of insulin

After the basal perfusion pressure had been allowed to stabilize, preparations were perfused with Krebs solution containing guanethidine (5 μM) to block adrenergic neurotransmission and methoxamine (2–7 μM) to induce submaximal contraction. After stabilization of the elevated perfusion pressure, the mesenteric vascular beds were subjected to perfusion of insulin. The final concentration of insulin was made by dilution of Krebs solution containing methoxamine plus guanethidine and perfused. The perfusion of insulin at concentrations of 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000 and 3000 nM was sequentially applied upon perfusion pressure stabilization. Vasodilator activity of the insulin is expressed as the pD_2 value, defined as the negative logarithm of the molar concentration producing 50% of the maximum response. At the end of each experiment, each preparation was perfused with 100 μM papaverine to cause complete relaxation. Vasodilator activity is expressed as a percentage of the perfusion pressure at maximum relaxation induced by papaverine.

Chemical removal of vascular endothelium

To remove the vascular endothelium, preparations with resting tone were perfused with a 1.80 mg ml^{-1} solution of sodium deoxycholate (SD) in saline for 30 s (Takenaga *et al*., 1995). This caused a transient increase (10–20 mmHg) in perfusion pressure. The preparations were rinsed with SD-free Krebs solution for 10 min and then perfused with Krebs solution for 30 min. After the preparations were contracted by perfusion with Krebs solution containing methoxamine and guanethidine, chemical removal of the endothelium was assessed by the lack of a relaxant effect after a bolus infusion of 1 nmol acetylcholine (ACh). ACh was infused directly into the perfusate proximal to the arterial cannula at 100 μl 10 s^{-1}, by an infusion pump (model 975, Harvard Apparatus, South Natick, Mas., U.S.A.).

Experimental protocol

To assess the underlying mechanism of the vasodilator effect of insulin, the effects of various agents were examined. An active tone of the preparation was produced by methoxamine (7 μM) and guanethidine (5 μM), and after the elevated perfusion pressure stabilized, Krebs solution containing the final concentration of insulin and 100 nM atropine (muscarinic cholinoceptor antagonist) plus 100 nM propranolol (β-adrenoceptor antagonist), 100 μM L-N^G-nitroarginine (nitric oxide synthase inhibitor, L-NOARG), 1 μM ouabain (Na^+-K^+ ATPase inhibitor) or 1 μM glibenclamide (ATP-sensitive K^+-channel inhibitor) was perfused. Doses of L-NOARG (Takenaga *et al*., 1995), ouabain (Weiss *et al*., 1993) and glibenclamide (McPherson & Angus, 1991) were chosen to inhibit selectively the channel and enzymes.

For the endothelium removal, an active tone in the mesenteric vascular beds with intact endothelium was produced by use of methoxamine (7 μM) and guanethidine (5 μM), followed by a bolus infusions of ACh (1 nmol). Subsequently, a methoxamine-free Krebs solution was used to rinse the preparation for 20 to 30 min, until the perfusion pressure returned to the control level. Then, chemical removal of the endothelium with SD was performed. After endothelium removal, the active tone of the preparation was reproduced by perfusion with Krebs solution containing methoxamine (3 μM) and guanethidine (5 μM). Subsequently, successful removal of vascular endothelium was confirmed by the lack of a relaxant effect after 1 nmol ACh infusion. Thereafter, vascular responses to perfusion of insulin in the vascular bed were examined.

The effects of capsaicin (1 μM), a toxin against peptidergic and sensory neurones, and human CGRP[8–37], a CGRP receptors antagonist, on the vasodilator response to insulin were examined in preparations without endothelium. The preparations were perfused with Krebs solution containing capsaicin (1 μM) for 20 min, rinsed with capsaicin-free Krebs solution for 20 min, and endothelium removal with SD. After being rinsed for 60 min the preparations were contracted by perfusion with Krebs solution containing methoxamine (2 μM) and guanethidine (5 μM). After the elevated perfusion pressure stabilized, periarterial nerve stimulation (PNS, 1 Hz) was performed to check for the presence of CGRP-containing nerves. PNS was applied for 30 s with bipolar platinum ring electrodes placed around the superior mesenteric artery. Rectangular pulses of 1 ms and supramaximal voltage (60 V) were applied at 1 Hz by using an electronic stimulator (model SEN 3301, Nihon Kohden). Successful denervation of CGRP-containing nerves and successful removal of vascular endothelium was confirmed by the lack of a relaxant effect to PNS (1 Hz) or ACh (1 nmol) infusion.

In another series of experiments, the effects of human CGRP[8–37] on insulin-induced responses were examined in preparations without endothelium. The Krebs solution containing methoxamine (2 μM), guanethidine (5 μM), each concentration of insulin and CGRP[8–37] (0.5 μM) was perfused into the preparation and upon vasopressure stabilization, a higher concentration of insulin was perfused.

Statistical analysis

Experimental results are presented as mean ± s.e.mean. Statistical analysis was performed by use of one-way analysis of variance (ANOVA) followed by Student's t test for two groups. ANOVA followed by Dunnett's test was used to determine the significance of the difference between groups. A P value less than 0.05 was considered statistically significant.

Drugs

The following drugs were used: ACh chloride (Daiichi Pharmaceutica, Tokyo, Japan), guanethidine sulphate (Tokyo

Kasei, Tokyo, Japan), methoxamine hydrochloride (Nihon Shinyaku, Kyoto, Japan), atropine sulphate (Fuso Chemical Co., Osaka, Japan), human insulin (Eli Lilly Japan, Kobe, Japan), propranolol hydrochloride (Zeneca Co., Osaka, Japan), ouabain (Aldrich Japan, Tokyo, Japan), glibenclamide (Sigma Chemical Co., St. Louis, Mo., U.S.A.), L-NOARG (Sigma), capsaicin (Sigma), rat CGRP (Peptide Institute, Osaka, Japan), human CGRP[8–37] (Peptide Institute) and papaverine hydrochloride (Dainippon Pharmaceutical, Osaka, Japan), sodium deoxycholate (Wako pure chemical industries, Osaka, Japan). All drugs were dissolved in distilled water and diluted with Krebs solution containing 5 to 7 μM methoxamine and 5 μM guanethidine, when perfused or infused directly. Sodium deoxycholate was dissolved in 0.9% saline.

Results

Vascular responses to perfusion of insulin in mesenteric resistance blood vessels of rats

In perfused mesenteric vascular beds, perfusion of Krebs solution containing 7 μM methoxamine and 5 μM guanethidine increased perfusion pressure, which was maintained throughout the experiment (Figure 1a). As shown in Figure 1b, the perfusion of insulin decreased perfusion pressure, in a concentration-dependent manner, due to vasodilatation. The pD_2 value was 6.94 ± 0.22, and maximum relaxation (% Max, expressed as % of the maximum relaxation induced by papaverine) was $43.9 \pm 5.2\%$ (Figure 1b and Table 1).

Effects of various drugs on vasodilator responses to perfusion of insulin

To assess the possible mechanisms underlying the vasodilator response to perfusion of insulin, the effects of the addition of propranolol, atropine, L-NOARG, ouabain, or glibenclamide were examined. As shown in Table 1, insulin-induced vasodilatation was not altered by the combined presence of 100 nM propranolol plus 100 nM atropine, which inhibited significantly vasodilator responses to a bolus infusion of 1 nmol isoprenaline (control response, $55.0 \pm 7.5\%$; response in the presence of antagonists, $14.1 \pm 1.8\%$, $n=5$, $P<0.01$) and 1 nmol ACh (control response, $81.9 \pm 1.9\%$; response in the presence of antagonists, $4.2 \pm 2.9\%$, $n=5$, $P<0.01$). In addition, neither 100 μM L-NOARG, 1 μM ouabain, nor 1 μM glibenclamide had an effect on the insulin-induced response (Table 1). There was no significant difference in both the pD_2 value and maximum response between the control preparations and preparations treated with various drugs.

Effect of chemical removal of vascular endothelium on vasodilator response to perfusion of insulin

Chemical removal of the vascular endothelium increased vasoconstriction induced by methoxamine. Therefore, the concentration of methoxamine used to raise the tone was reduced to 3 μM in denuded beds. Chemical removal of the endothelium abolished the vasodilator response to a bolus infusion of 1 nmol ACh, indicating that the endothelium was successfully removed (Figure 2).

In the preparation without endothelium, perfusion of insulin produced a marked vasodilatation in a concentration-dependent manner (Figure 2). The insulin-induced vasodilatation was significantly greater in preparations without en-

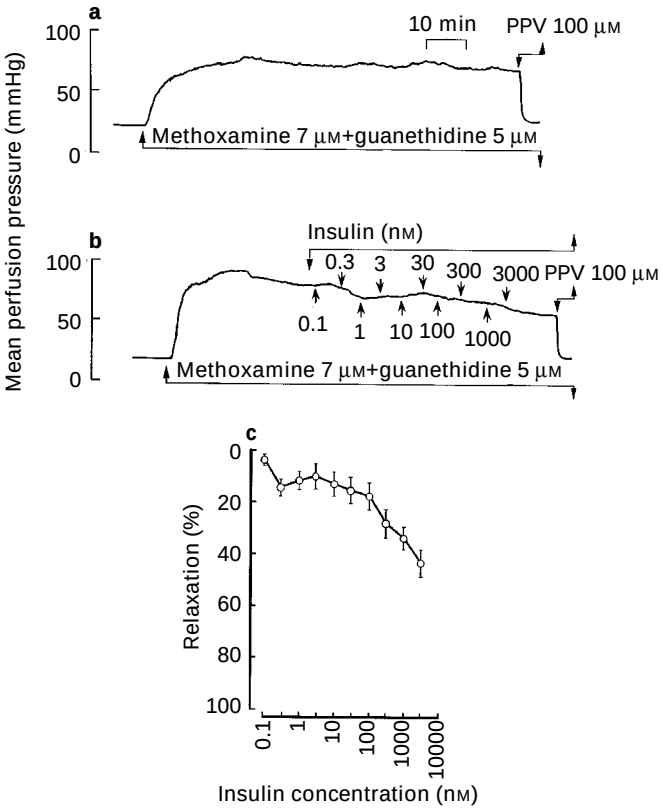

Figure 1 Typical records showing changes in perfusion pressure in the absence (a) and presence (b) of insulin and graph (c) showing vasodilator response to insulin in rat perfused mesenteric vascular beds with active tone produced by 7 μM methoxamine plus 5 μM guanethidine. (c) Relaxation (%) indicates the percentage of the perfusion pressure at maximum relaxation induced by 100 μM papaverine. Each value shows the mean and vertical lines s.e.mean. PPV, papaverine perfusion.

Table 1 Effects of various treatments on pD_2 values and maximum vasorelaxation induced by insulin in rat mesenteric resistance vessels with active tone produced by methoxamine and guanethidine

Treatments	(n)	*Concentrations*	*pD_2 ($-$logM)*	*% Max*
Intact	(6)		6.94 ± 0.22	43.9 ± 5.2
Atropine + propranolol	(5)	100 nM + 100 nM	7.10 ± 0.29	53.9 ± 4.2
L-NOARG	(4)	100 μM	7.28 ± 0.33	61.9 ± 7.7
Ouabain	(5)	1 μM	7.48 ± 0.36	53.6 ± 9.5
Glibenclamide	(5)	1 μM	7.98 ± 0.75	56.8 ± 6.6

Values are mean $\pm$ s.e.mean. pD_2 negative logarithm of the molar concentration of 50% effective dose; % Max, maximum relaxation induced by insulin expressed as % of the maximum relaxation induced by 100 μM papaverine; (n), number of animals.

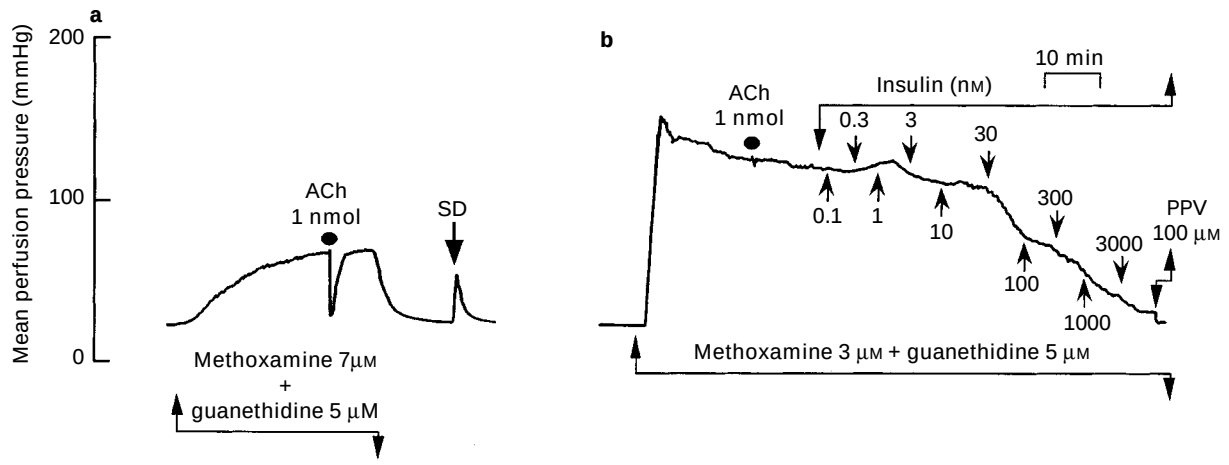

Figure 2 Typical records showing effect of endothelium removal on vasodilator response to insulin in perfused mesenteric vascular beds with active tone. (a) Control response in the preparation with intact endothelium. (b) Responses in the preparation without endothelium. SD, perfusion of sodium deoxycholate. PPV, papaverine perfusion.

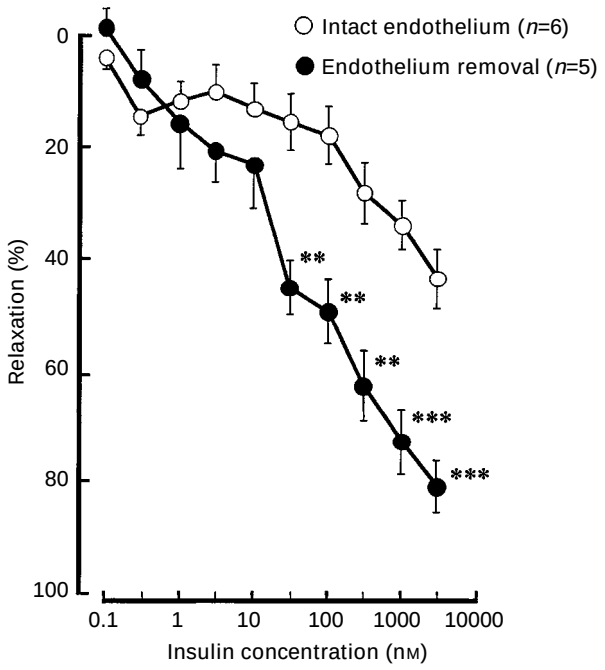

Figure 3 Effect of endothelium removal on vasodilator responses to insulin in rat perfused mesenteric vascular beds with active tone produced by methoxamine and guanethidine. Relaxation (%) indicates the percentage of the perfusion pressure at maximum relaxation induced by 100 μM papaverine. Each value shows the mean and vertical lines indicate s.e.mean. $^{**}P<0.01$, $^{***}P<0.001$, compared with intact endothelium.

Table 2 pD_2 values and maximum relaxation (% Max) of insulin-induced vasodilatation in perfused mesenteric vascular beds with or without endothelium

	(n)	pD_2 ($-logM$)	% Max
Endothelium-intact	(6)	7.06±0.36	42.9±5.6
Endothelium-removed	(5)	7.62±0.21	81.0±4.6*

Values are mean±s.e.mean. pD_2, negative logarithm of the molar concentration of 50% effective dose; % Max, maximum relaxation induced by insulin expressed as % of the maximum relaxation induced by 100 μM papaverine. $^*P<0.001$ vs endothelium-intact. (*n*), number of animals.

Table 3 Effects of treatment with capsaicin or calcitonin gene-related peptide [8–37] (CGRP 8–37) on pD_2 values and maximum relaxation induced by insulin in rat perfused mesenteric resistance vessels without endothelium

Treatments	(n)	pD_2 ($-logM$)	% Max
Control (endothelium-removed)	(5)	7.62±0.21	81.0±4.6
Capsaicin (1 μM)	(6)	7.47±0.34	71.8±4.2
CGRP [8–37] (0.5 μM)	(4)	6.56±0.14**	59.2±3.9*

Values are mean±s.e.mean. pD_2, negative logarithm of the molar concentration of 50% effective dose; % Max, maximum relaxation induced by insulin expressed as % of the maximum relaxation induced by 100 μM papaverine. $^*P<0.05$, $^{**}P<0.001$, compared with control. (*n*), number of animals.

dothelium than in those with intact endothelium (Figure 3). The maximum relaxation responses induced by insulin in denuded preparations were significantly greater than in intact preparations (Table 2).

Effects of capsaicin and human CGRP[8–37] on vasodilator responses to perfusion of insulin in preparations without endothelium

In preparations without endothelium, capsaicin treatment abolished vasodilatation in response to PNS, indicating successful denervation of CGRP nerve as shown previously (Kawasaki *et al*., 1988; 1990a; Takenaga *et al*., 1995). As indicated in Table 3, treatment with capsaicin did not affect insulin-induced vasodilatation.

The perfusion of Krebs solution containing 0.5 μM CGRP[8–37] resulted in a transient increase in mean perfusion pressure (Figure 4) and it attenuated the vasodilator response both to a bolus infusion of CGRP (100 pmol) (control response, 72.9±15.4%; response in the presence of CGRP[8–37], 3.1±1.7%, $P<0.05$, $n=3$) and to PNS (1 Hz) (control response, 55.1±6.3%; response in the presence of CGRP[8–37], 2.6±2.5%, $P<0.005$, $n=3$) (Figure 4a). Treatment with CGRP[8–37] at a dose of 0.5 μM inhibited

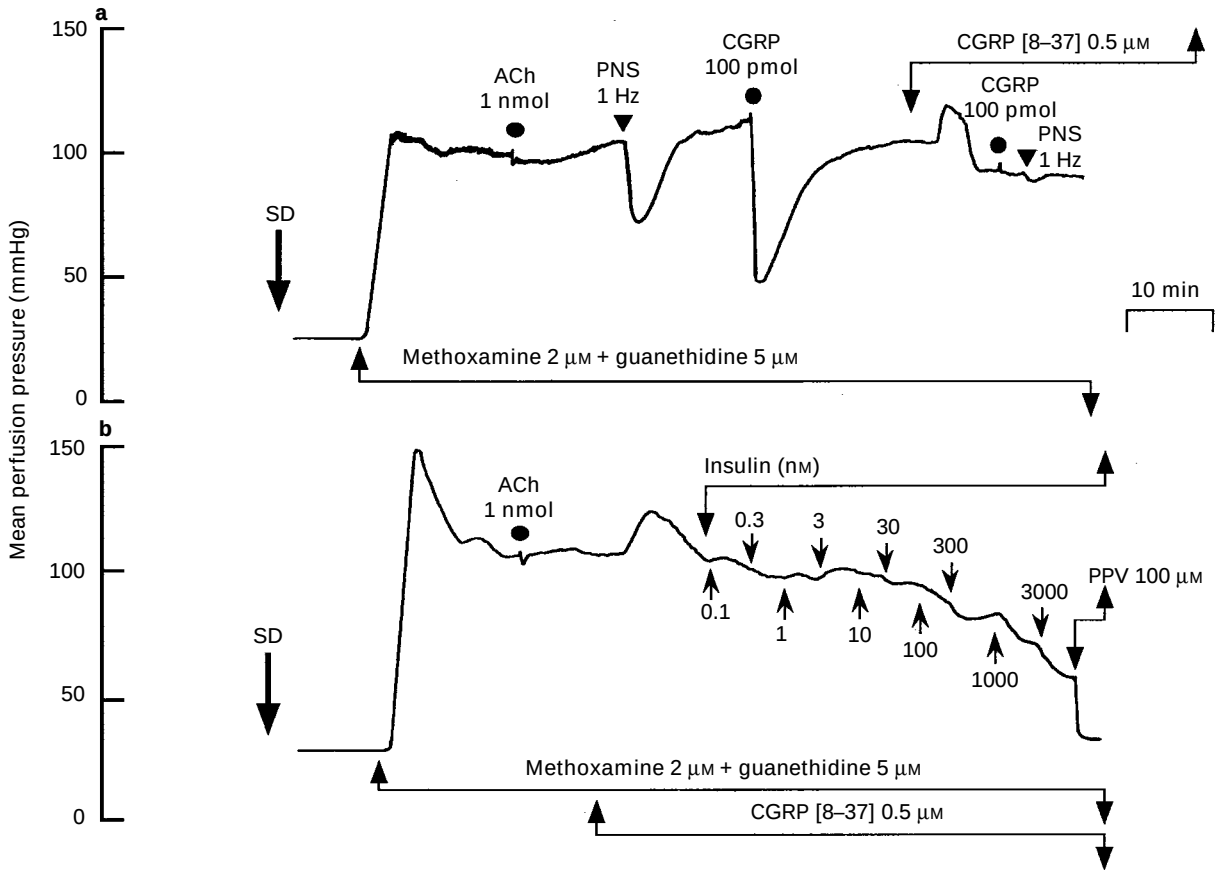

Figure 4 Typical records of the effect of human calcitonin gene-related peptide[8–37] (CGRP[8–37]) treatment on vasodilator responses to infusion of acetylcholine (ACh, 1 nmol), CGRP (100 pmol), periarterial nerve stimulation (PNS; 1 Hz), or insulin perfusion in the rat perfused mesenteric vascular beds without endothelium. SD, perfusion of sodium deoxycholate. PPV, papaverine perfusion.

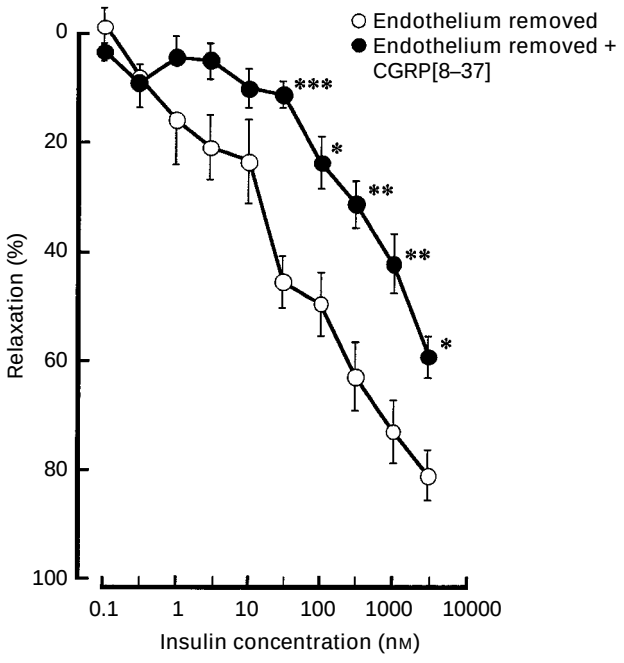

Figure 5 Effect of human calcitonin gene-related peptide[8–37] (CGRP [8–37]) on vasodilator responses to insulin in rat perfused mesenteric vascular beds with active tone produced by methoxamine and guanethidine. Relaxation (%) indicates the percentage of the perfusion pressure at maximum relaxation induced by 100 μM papaverine. Each value shows the mean and vertical lines indicate s.e.mean. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared with control endothelium denuded preparations.

the vasodilator response to insulin (Figure 4 and Figure 5). There were significant differences in the pD_2 value and maximum relaxation between preparations treated with CGRP[8–37] and untreated preparations (Figure 5 and Table 3).

Discussion

The present study demonstrated that insulin causes a concentration-dependent vasodilator response in mesenteric resistance blood vessels with active tone. The vasodilator action of insulin was not affected by the combined presence of propranolol (β-adrenoceptor antagonist) and atropine (muscarinic cholinoceptor antagonist) at a dose that abolished vasodilatation induced by isoprenaline (β-adrenoceptor agonist) and ACh (cholinoceptor agonist). Thus, it is likely that the vasodilatation induced by insulin in the mesenteric resistance blood vessel is non-adrenergic, non-cholinergic in nature.

Insulin has been shown to cause vasodilatation in large arteries isolated from several species including man (Creager *et al*., 1985; Laakso *et al*., 1990), dog (Liang *et al*., 1982) and rat (Gros *et al*., 1994). Several mechanisms underlying insulin-induced vasodilatation have been proposed. In the human forearm (Ferrannini *et al*., 1988) and vascular smooth muscle cells (Prakash *et al*., 1992), insulin causes the activation of Na^+-K^+ ATPase which decreases intracellular Na^+ and then Ca^{2+}. Also, insulin opens ATP-sensitive K^+-channels,

hyperpolarizing the membrane potential. In the present study, insulin-induced vasodilatation was not affected by treatment with ouabain, a Na^+-K^+ ATPase inhibitor, or glibenclamide, an ATP-sensitive K^+-channel blocker. Therefore, it is unlikely that Na^+-K^+ ATPase activation and ATP-sensitive K^+-channel opening are involved in insulin-induced vasodilatation in the mesenteric resistance artery.

In human lower limb arteries (Steinberg *et al*., 1993) and dog arteries (D'Orleans-Juste *et al*., 1985), insulin-induced vasodilatation has been shown to be inhibited by endothelium removal or by a nitric oxide (NO) synthase inhibitor, suggesting that the vasodilatation is endothelium-dependent and is mediated by NO. However, the present study indicates that insulin-induced vasodilatation is not inhibited and, in fact, is markedly potentiated by endothelium removal. Furthermore, the NO synthase inhibitor, L-NOARG, had no effect on insulin-induced vasodilatation. These results strongly suggest that insulin-induced vasodilatation in the mesenteric resistance blood vessel is endothelium- and NO-independent.

The removal of the endothelium in the mesenteric resistance vessel markedly potentiated the vasodilator responses to insulin, indicating that the endothelium masks insulin-induced vasodilatation. It is well known that the endothelium releases endothelium-derived contracting factors (EDCF) such as endothelin and prostaglandins. Thus, it is possible that insulin triggers endothelial cells to release EDCF and therefore decrease vasodilatation. Another possibility is that endothelium removal causes greater access of insulin to the underlying smooth muscle cells. However, further study is needed to clarify this mechanism.

We have shown that the rat mesenteric resistance blood vessel is innervated by CGRP-containing vasodilator nerves (Kawasaki *et al*., 1988; 1990a,b). Periarterial nerve stimulation of the preparation produces a neurogenic vasodilator response and the release of CGRP into the perfusate. Previous studies have shown that human CGRP[8–37], which acts as a selective and competitive antagonist of CGRP receptors, inhibits the neurogenic vasodilator response (Han *et al*., 1990; Takenaga *et al*., 1995), indicating that the response is mediated by endogenous CGRP that is released from CGRP-containing vasodilator nerves. In the present study, vasodilatation induced by insulin in the preparation without endothelium was significantly inhibited by CGRP[8–37] at a dose that abolished both CGRP infusion- and PNS-induced vasodilatation. These results strongly suggest that the endothelium independent vasodilatation induced by insulin in the mesenteric resistance blood vessel is partially mediated by activation of CGRP receptors.

Insulin is a 51 amino acid peptide and consists of 2 chains of amino acids, A and B, connected by two disulphide bridges. The A and B chains are composed of 21 and 30 amino acid residues, respectively. The A chain has a disulphide bridge between amino acids 6 and 11, including five amino acids and are corresponding to position 2 and 7 of amino sequence of CGRP with the same number of amino acid residues. The disulphide bridge at position 2 and 7 of CGRP is an important region for vasodilator activity (Nuki *et al*., 1994). Therefore, it is likely that a disulphide bridge between position 6 and 11 of A chain in insulin may be responsible for the vasodilator activity at the CGRP receptor site. A novel vasodilator peptide, adrenomedullin, has 20% homologous amino acid sequence to CGRP, they share a ring structure with a disulphide bridge that includes 5 amino acid residues (Kitamura *et al*., 1993). Previously, it was shown that the vasodilator response to adrenomedullin was markedly inhibited by CGRP[8–37] (Nuki *et al*., 1993), suggesting that adrenomedullin-induced vasodilatation is mediated by CGRP receptors. These results, taken together, strongly suggest that a disulphide bridge containing five amino acids is essential for CGRP-receptor mediated vasodilatation. However, further study is needed to clarify the structure-activity relationship between these peptides.

In conclusion, the present study suggests that insulin induces endothelium-independent vasorelaxation in the mesenteric resistance blood vessel, and that CGRP receptors are involved in this vasorelaxation.

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