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Insulin-induced relaxation of rat mesenteric artery is mediated by Ca^{2+}-activated K^+ channels

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Abstract

We tested the hypothesis that relaxation of the rat mesenteric artery in response to insulin is mediated by K^+ channels. Two concentrations of insulin (10 and 100 mU/ml) induced relaxation of the artery by 6 ± 1%, 24 ± 3% (mean ± S.E.M.). Denudation of the endothelium or precontraction by KCl (30 mM), clotrimazole (10 μM), a cytochrome P450 inhibitor, charybdotoxin (30 nM) an inhibitor of large-conductance Ca^{2+}-activated K^+ channels, abolished the relaxation of the artery in response to insulin. However, N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 μM), an inhibitor of nitric oxide synthase, apamin (1 μM), an inhibitor of small-conductance Ca^{2+}-activated K^+ channels, or glibenclamide (10 μM), an ATP-sensitive K^+ channels blocker, did not attenuate the relaxation of the artery caused by insulin. These results suggest that the relaxation of rat mesenteric artery in response to insulin is mediated mostly by large-conductance Ca^{2+}-activated K^+ channels, perhaps an endothelium-derived hyperpolarizing factor (EDHF).

Keywords: Insulin; Vasorelaxation; K^+ channels Ca^{2+}-activated; EDHF (endothelium-derived hyperpolarizing factor); P450 metabolite

1. Introduction

Insulin has important cardiovascular effects, and hyperinsulinemia has been recognized recently as an important cardiovascular risk factor. There are a variety of reports of the vascular effects of insulin. Many studies, however, suggest that acute administration of insulin induces vasorelaxation, and that this relaxation in some vessels is mediated by nitric oxide (NO) (Scherrer et al., 1994; Steinberg et al., 1994).

Recent studies indicate that the relaxation of small mesenteric arteries in response to acetylcholine may be mediated in part by endothelium-derived hyperpolarizing factor (EDHF) (Shimokawa et al., 1996). EDHF may play an important role in the relaxation of mesenteric vessels. In response to EDHF, there is an increase in smooth muscle K^+ conductance which can be abolished by raising the external K^+ concentration (Chen et al., 1991; Nagao and Vanhoutte, 1991; Taylor and Weston, 1988).

The aim of the present study was to test the hypothesis that insulin-induced relaxation of rat mesenteric artery is mediated by K^+ channels. We tested whether charybdotoxin and apamin, which are inhibitors of Ca^{2+}-activated K^+ channels (Blatz and Magleby, 1986; Langton et al., 1993; Miller et al., 1985), and glibenclamide, which is an ATP-sensitive K^+ channels blocker, inhibit vasorelaxation of the mesenteric artery in response to insulin.

2. Materials and methods

2.1. Preparation of vascular rings

Male Sprague–Dawley rats, 12–14 weeks old, were obtained from Charles River Japan (Yokohama). The mesentery was removed from Sprague–Dawley rats under ether anesthesia. The entire mesenterium was removed and placed in cold modified Krebs–Ringer bicarbonate solution of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 12: pH 7.4 (Krebs solution). The first-order branch of the mesenteric artery was carefully dissected and cleaned of adhering adipose tissue and cut into a ring

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(external diameter, 400–450 μm; length, 3 mm) under a dissection microscope.

This ring was suspended horizontally using two L-shaped stainless steel holders attached to the lumen in a small chamber (5 ml) filled with Krebs solution (37°C) bubbled with a mixture of 95% O_2 and 5% CO_2. One holder was fixed and the other was connected to a force-displacement transducer (UM-203, Kishimoto, Kyoto) to measure isometric force. The ring was equilibrated under a resting force of 500 mg for 60 min. This force was found to be optimal for contraction of the ring, as assessed by repeated exposure to KCl (90 mM) at various resting forces. After the equilibration period, each ring was maximally contracted twice by phenylephrine to examine the reproducibility of contraction.

Concentration–response curves for phenylephrine were obtained in preliminary experiments, and the concentration of phenylephrine that produced 50% of the maximum contractile response (EC_{50}) was used in subsequent studies. Results are expressed as percent relaxation of the maximum contraction produced by phenylephrine.

2.2. Experimental protocol

To determine the effects of insulin, after the contraction elicited by phenylephrine reached a plateau, we measured the change in vascular tension in response to three concentrations (1, 10, and 100 mU/ml) of insulin. We also examined the effects of removing the endothelium on the vasorelaxation induced by insulin. To remove the endothelium, we gently rolled a stainless steel wire in the intimal surface and examined responses to acetylcholine (1 nM–10 μM).

To examine the role of NO and K^+ channels, we examined the effects on the relaxation in response to insulin of N^{ω}-nitro-L-arginine methyl ester (L-NAME;100 μM), an inhibitor of nitric oxide synthase (Rees et al., 1990), and several K^+ channel blockers. We examined the effects of charybdotoxin (30 nM), a specific blocker of large-conductance Ca^{2+}-activated K^+ channels (Miller et al., 1985), apamin (1 μM), a specific blocker of small-conductance Ca^{2+}-activated K^+ channels (Blatz and Magleby, 1986), and glibenclamide (10 μM), an ATP-sensitive K^+ channels blocker (Quast and Cook, 1989). L-NAME was applied 30 min before exposure to insulin and did not affect the phenylephrine-induced contraction. Charybdotoxin, apamin and glibenclamide were applied 15 min before exposure to insulin.

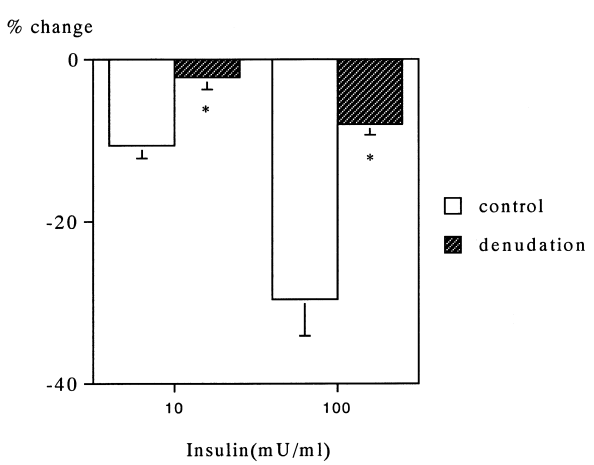

Fig. 1. Effects of endothelial denudation on insulin-induced relaxation of mesenteric artery. Open column is control group, and shaded column is denudation group. Values are means ± S.E.M., $n = 6$. $^*P < 0.05$, vs. control group.

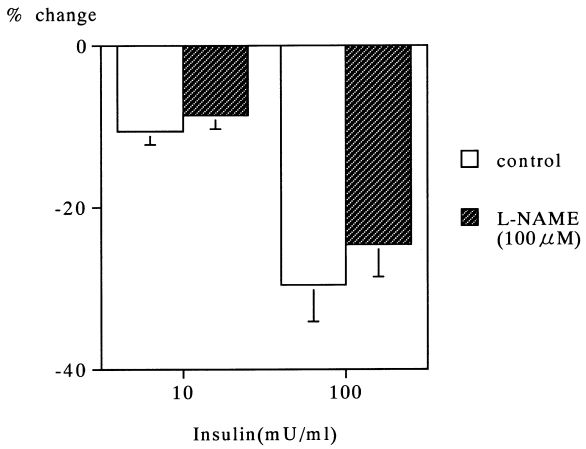

Fig. 2. Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME) (10 μM) on insulin-induced relaxation of mesenteric artery. Open column is control group, and shaded column is L-NAME group. Values are means ± S.E.M., $n = 7$.

In additional experiments, we examined the responses to insulin in the depolarized mesenteric artery. After the contraction of the artery elicited by KCl (30 mM) alone had reached a plateau, we measured the change in vascular force in response to insulin.

To examine the effect of a putative EDHF inhibitor, we examined the effect of clotrimazole (10 μM), a cytochrome P450 inhibitor (Hecker et al., 1994), on the relaxation of mesenteric artery caused by insulin. Clotrimazole was applied 15 min before insulin induced vasorelaxation.

The following drugs were used: phenylephrine, acetylcholine, sodium nitroprusside, L-NAME, apamin, glibenclamide (from Sigma, St. Louis, MO, USA), clotrimazole (from Research Biochemicals, Natick, MA, USA), and charybdotoxin (from Latoxan, Rosana, France). Phenylephrine, acetylcholine, sodium nitroprusside, and L-NAME were dissolved in Krebs solution. Charybdotoxin and apamin were dissolved in distilled water. Glibenclamide was dissolved in dimethyl sulfoxide (DMSO) and clotrimazole was dissolved in ethanol. DMSO vehicle (0.1%) alone or ethanol vehicle alone did not inhibit the relaxation elicited by insulin.

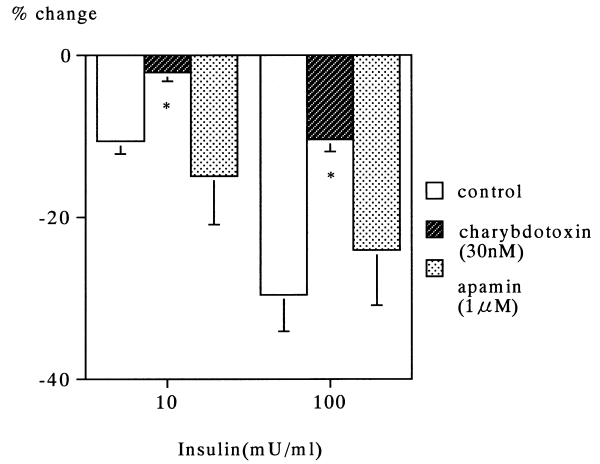

Fig. 3. Effects of charybdotoxin (30 nM) and apamin (1 μM) on insulin-induced relaxation of mesenteric artery. Open column is control group, and shaded column is charybdotoxin group, and dotted column is apamin group. Values are means ± S.E.M., $n = 6$. * $P < 0.05$, vs. control group.

2.3. Statistical analysis

All data are expressed as means ± S.E.M.. Student's *t*-test was used to compare absolute values, and the Mann–Whitney *U*-test was used to compare percent changes. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Effects of endothelial denudation on relaxation in response to insulin

Insulin produced a concentration-dependent vasorelaxation in the presence of phenylephrine. Insulin (10 mU/ml) produced relaxation of the mesenteric artery by 6 ± 1%, and a higher concentration of insulin (100 mU/ml) produced relaxation by 24 ± 3%. However, insulin (1 mU/ml) did not produce relaxation of the mesenteric artery. Removal of the endothelium abolished the relaxation of the mesenteric artery in response to insulin (Fig. 1).

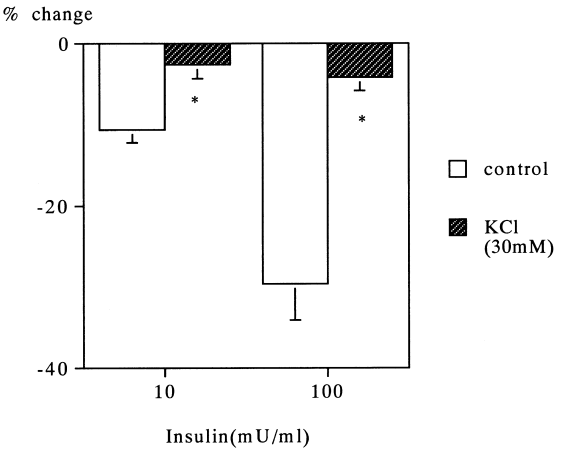

Fig. 5. Effects of KCl (30 mM) on insulin-induced relaxation of mesenteric artery. Open column is control group, and shaded column is KCl group. Values are means ± S.E.M., $n = 7$. * $P < 0.05$, vs. control group.

3.2. Effects of L-NAME, K^+ channel blockers, and clotrimazole

The resting force of the mesenteric artery did not change in the presence of insulin, L-NAME, charybdotoxin, apamin, glibenclamide or clotrimazole.

The relaxation of the mesenteric artery elicited by insulin was not attenuated by L-NAME (Fig. 2). This concentration of L-NAME eliminated the acetylcholine-induced relaxation of the mesenteric artery, without the affecting the vasorelaxation in response to sodium nitroprusside. Charybdotoxin abolished the insulin-induced va-

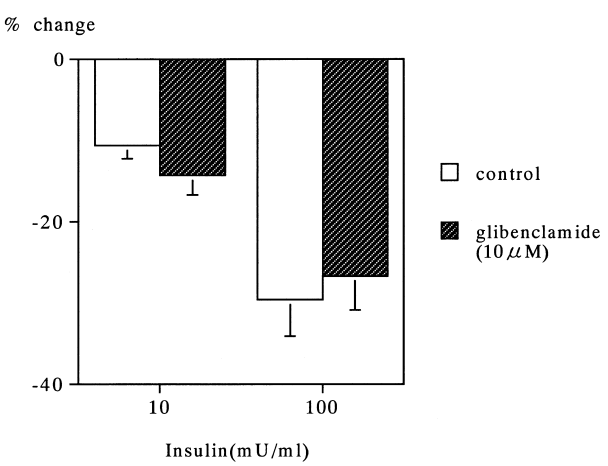

Fig. 4. Effects of glibenclamide (10 μM) on insulin-induced relaxation of mesenteric artery. Open column is control group, and shaded column is glibenclamide group. Values are means ± S.E.M., $n = 8$.

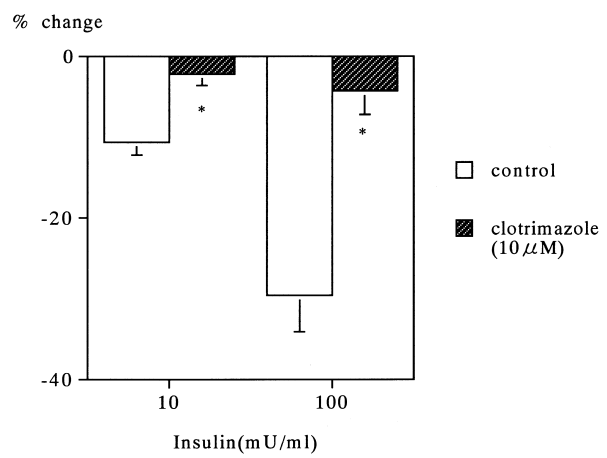

Fig. 6. Effects of clotrimazole (10 μM) on insulin-induced relaxation of mesenteric artery. Open column is control group, and shaded column is clotrimazole group. Values are means ± S.E.M., $n = 7$. * $P < 0.05$, vs. control group.

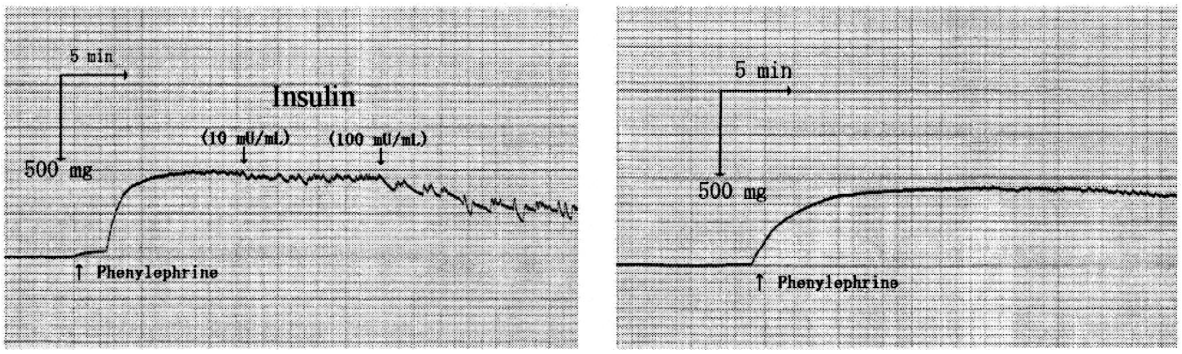

Fig. 7. The left panel shows actual recording of the contraction induced by an EC_{50} concentration of phenylephrine in rat mesenteric artery without insulin, and the right panel shows the effect of cumulative application of insulin (10 and 100 mU/ml) on the phenylephrine-induced contraction.

sorelaxation (Fig. 3), whereas neither apamin (Fig. 3) nor glibenclamide affected the insulin-induced vasorelaxation (Fig. 4). Insulin did not induce relaxation of precontracted mesenteric arteries in the presence of KCl (Fig. 5), and clotrimazole abolished the insulin-induced vasorelaxation (Fig. 6). Actual recordings of the cumulative effect of insulin on the phenylephrine-induced contraction and of the effect of phenylephrine alone in the rat mesenteric artery are shown in Fig. 7.

4. Discussion

The major new finding of the present study is that the relaxation of the mesenteric artery from rats in response to insulin is mediated by large-conductance Ca^{2+}-activated K^+ channels, is abolished by endothelial denudation, and thus may be mediated by EDHF. Numerous studies have shown that insulin induces relaxation of human coronary, pulmonary and radial arteries (Anderson et al., 1991; Sasaki et al., 1993) and inhibits the vasocontraction produced by norepinephrine, phenylephrine and angiotensin II (Grover et al., 1995). Possible mechanisms by which insulin induces vasorelaxation are endothelium-dependent, -independent or both (D'Orleans-Juste et al., 1985; Han et al., 1995; Thom et al., 1988). It is also suggested by act by an α,β-adrenergic mechanism (Creager et al., 1985), stimulation of plasma membrane Ca^{2+}-ATPase (Zemel et al., 1992), and inhibition of Ca^{2+} channels (Kahn et al., 1993; Standley et al., 1991).

We observed dose-dependent relaxation of the mesenteric artery in rats in response to insulin, and removal of the endothelium almost eliminated the relaxation induced by insulin. These results suggest that insulin-induced vasorelaxation is mediated by an endothelium-derived relaxing factor (EDRF). The endothelium releases several vasodilator substances, including prostacyclin, NO and possibly EDHF. One mechanism for vasorelaxation is activation of guanylate cyclase and increased production of cGMP (Rembold, 1992). EDRF, which appears to be NO or a related compound (Moncada et al., 1991), is an endogenous stimulus that activates guanylate cyclases and thereby causes vasorelaxation. A second mechanism for vasorelaxation is activation of adenylate cyclase and production of cAMP. The vasorelaxation produced by prostacyclin is mediated by formation of cAMP (Gryglewski et al., 1991).

K^+ channels are present in the mesenteric artery and activation of these channels may be a third mechanism for vasorelaxation. The membrane potential of vascular muscle is an important determinant of vascular tone, and the activity of K^+ channels is a major regulator of membrane potential. Activation of these channels increases K^+ efflux, producing hyperpolarization of vascular muscle. Membrane hyperpolarization closes voltage-dependent Ca^{2+} channels and thereby causes relaxation of vascular muscle (Bonnet et al., 1991; Nelson, 1993; Nelson and Quayle, 1995).

We determined whether the relaxation of the mesenteric artery in response to insulin is mediated by NO. Studies of several vessels have demonstrated that insulin produces vasodilatation by releasing of endothelium-derived nitric oxide (EDNO). Intra-arterial infusion of N^G-monomethyl-L-arginine (L-NNA) completely eliminates insulin-induced vasodilation in the forearm during systemic insulin infusion (Scherrer et al., 1994; Steinberg et al., 1994). In the present study, L-NAME did not attenuate insulin-induced vasorelaxation. One difference in these studies may relate to the size of the vessels. The importance of EDHF appears to increase as the vessel size decreases in the rat mesenteric circulation. Another difference between the studies may relate to the vascular bed that is studied. In the rat isolated mesenteric arterial bed, muscaranic receptor-induced vasorelaxation is mediated by both NO-dependent and -independent mechanisms (Han et al., 1995). The NO-independent mechanism probably occurs via activation of a K^+ conductance, with characteristics of EDHF-mediated responses (Han et al., 1995). We therefore examined the effects of K^+ channel blockers on the relaxation of the rat mesenteric artery in response to insulin.

EDHF is an as yet unidentified substance that hyperpolarizes vascular smooth muscle cells and produces relaxation. In bioassay experiments, endothelium-dependent hy-

perpolarizing activity can be transferred from tissues with endothelium to those without endothelium, indicating the existence of a factor of endothelial origin that diffuses to the underlying vascular smooth muscle and produces hyperpolarization (Cambell et al., 1996; Hwa et al., 1994; McPherson and Angus, 1991). Endothelium-dependent hyperpolarization results from the opening of K^+ channels. Several types of K^+ channels, including Ca^{2+}-activated K^+ channels, ATP-sensitive K^+ channels, delayed rectifier K^+ channels, and inward rectifier K^+ channels, have been identified in blood vessels (Nelson and Quayle, 1995). We therefore used several types of K^+ channel blockers. Our results showed that charybdotoxin and KCl inhibited insulin-induced vasorelaxation, and that apamin and glibenclamide did not inhibit the relaxation significantly. These results suggest that large-conductance, Ca^{2+}-activated K^+ channels, and not ATP-sensitive K^+ channels (McPherson and Angus, 1991) have an important role in insulin-induced relaxation of the mesenteric arteries.

Recently, some articles suggested that EDHF activity may reflect the action of cytochrome P450-derived arachidonic acid metabolites (Cambell et al., 1996; Hecker et al., 1994). We therefore examined the effect of clotrimazole, an inhibitor of cytochrome P450, on insulin-induced relaxation of the mesenteric artery. Clotrimazole inhibited insulin-induced vasorelaxation as effectively as the blockers of Ca^{2+}-activated K^+ channels.

Prostacyclin is released from the endothelium, activates adenylate cyclase and produces vasorelaxation. In the present experiment, however, indomethacin, an inhibitor of the cyclooxygenase pathway, did not significantly change the insulin-induced vasorelaxation, suggesting that the release of prostacyclin is not responsible for the relaxation induced by insulin (data not shown). Other studies also suggest that indomethacin does not reduce the hyperpolarization induced by acetylcholine and that the effect of EDHF is not mediated by a cyclooxygenase pathway (Chen et al., 1988; Feletou and Vanhoutte, 1988).

In conclusion, the relaxation of the mesenteric artery in response to insulin is largely mediated by large-conductance, Ca^{2+}-activated K^+ channels, perhaps EDHF.

References

Anderson, E.A., Hoffman, R.P., Balon, T.W., Sinkey, C.A., Mark, A.L., 1991. Hyperinsulinemia produces both sympathetic neural activation and vasodilatation in normal humans. J. Clin. Invest. 87, 2246–2252.

Blatz, A.L., Magleby, K.L., 1986. Single apamin-blocked Ca^{2+}-activated K^+ channels of small conductance in cultured rat skeletal muscle. Nature 323, 718–720.

Bonnet, P., Rush, N.J., Harder, D.R., 1991. Characterization of an outward K^+ current in freshly dispersed cerebral arterial muscle cells. Pfluegers Arch. 418, 292–296.

Cambell, W.B., Gebremedhin, D., Pratt, P.F., Harder, D.R., 1996. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ. Res. 78, 415–423.

Chen, G., Suzuki, H., Weston, A.H., 1988. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br. J. Pharmacol. 95, 1165–1174.

Chen, G., Yamamoto, Y., Miwa, K., Suzuki, H., 1991. Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. Am. J. Physiol. 260, H1888–H1892.

Creager, M.A., Liang, C.S., Coffman, J.D., 1985. Beta-adrenergic mediated vasodilator response to insulin in the human forearm. J. Pharmacol. Exp. Ther. 235, 709–714.

D'Orleans-Juste, P., Dion, S., Mizrahi, J., Regoli, D., 1985. Effects of peptides and non-peptides on isolated arterial smooth muscle: role of endothelium. Eur. J. Pharmacol. 114, 9–21.

Feletou, M., Vanhoutte, P.M., 1988. Endothelium-dependent hyperpolarization of canine coronary artery smooth muscle. Br. J. Pharmacol. 93, 515–524.

Grover, A., Padington, C., Wilson, M.F., Sung, B.H., Izzo, J.F. Jr., Dandona, P., 1995. Insulin attenuates norepinephrine-induced venoconstriction: an ultrasonographic study. Hypertension 25, 779–784, (pt2).

Gryglewski, R.J., Botting, R.M., Vane, J.R., 1991. Prostacyclin: from discovery to clinical application. In: Rubani, G.M. (Ed.), Cardiovascular Significance of Endothelium-Derived Vasoactive Factors. Futura Publishing, Mount Kisco, NY, pp. 3–37.

Han, S., Ouchi, Y., Karaki, H., Orimo, H., 1995. Inhibitory effects of insulin on cytosolic Ca^{2+} level and contraction in the rat aorta: endothelium-dependent and-independent mechanisms. Circ. Res. 77, 673–678.

Hecker, M., Bara, A.T., Bauersachs, J., Busse, R., 1994. Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolite in mammals. J. Physiol. 481, 407–414.

Hwa, J.J., Ghibaudi, L., Williams, P., Chatterjee, M., 1994. Comparision of acetylcholine-dependent relaxation in large and small arteries of rat mesenteric vascular bed. Am. J. Physiol. 266, H952–H958.

Kahn, A.M., Seidel, C.L., Allen, J.C., O'Neil, R.G., Shelat, H., Song, T., 1993. Insulin reduces contraction and intracellular calcium concentration in vascular smooth muscle. Hypertension 22, 735–742.

Langton, P.D., Nelson, M.T., Huang, Y., Standen, N.B., 1993. Block of Ca^{2+}-activated K^+ channels in mammalian arterial myocytes by tetraethylammonium ions. Am. J. Physiol. 260, H927–H934.

McPherson, G.A., Angus, J.A., 1991. Evidence that acetylcholine-mediated hyperpolarization of the rat small mesenteric artery does not involved the K^+ channel opened by cromakalim. Br. J. Pharmacol. 103, 1184–1190.

Miller, C., Moczydlowski, E., Latorre, R., Phillips, M., 1985. Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. Nature 313, 316–318.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43, 109–142.

Nagao, T., Vanhoutte, P.M., 1991. Hyperpolarization contributes to endothelium-dependent relaxations to acetylcholine in femoral veins of rats. Am. J. Physiol. 261, H1034–H1037.

Nelson, M.T., 1993. Ca^{2+}-activated potassium channels and ATP-sensitive potassium channels as modulators of vascular tone. Trends Cardiovasc. Med. 3, 54–60.

Nelson, M.T., Quayle, J.M., 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268, C799–C822.

Quast, U., Cook, N.S., 1989. Moving together: K^+ channel openers and ATP-sensitive K^+ channels. Trends Pharmacol. Sci. 10, 431–435.

Rees, D.D., Palmer, R.M.J., Schulz, R., Hodson, H.F., Moncada, S., 1990. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746–752.

Rembold, C.M., 1992. Regulation of contraction and relaxation in arterial smooth muscle. Hypertension 20, 129–137.

Sasaki, K., Imaizumi, T., Masaki, H., Takeshita, A., 1993. Intra-arterial

infusion of insulin attenuates vasoreactivity in human forearm. Hypertension 22, 67–73.

Scherrer, U., Randin, D., Vollenweider, P., Vollenweider, L., Nicod, P., 1994. Nitric oxide release accounts for insulin's vascular effects in humans. J. Clin. Invest. 94, 2511–2515.

Shimokawa, S., Yasutake, H., Nagao, T., Egashira, K., Fujishima, M., Takeshita, A., 1996. The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. J. Cardiovasc. Pharmacol. 28, 703–711.

Standley, P.R., Zhang, F., Ram, L.J., Zemel, M.B., Sowers, J.R., 1991. Insulin attenuates vasopressin-induced calcium transients and a voltage dependent calcium response in rat vascular smooth muscle cells. J. Clin. Invest. 88, 1230–1236.

Steinberg, H.O., Brechtel, G., Johnson, A., Fineberg, N., Baron, A.D., 1994. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. J. Clin. Invest. 94, 1172–1179.

Taylor, S.G., Weston, A.H., 1988. Endothelium derived hyperpolarizing factor: an endogenous inhibitor released from the vascular endothelium. Trends Pharmacol. Sci. 9, 272–274.

Thom, S., Hughes, A., Sever, P.S., 1988. Endothelium-dependent responses in human arteries. In: Vanhoutte, P.M. (Ed.), Relaxing and Contracting Factors Clifton. Humana Press, Clifton, NJ.

Zemel, M.B., Johnson, B.A., Ambrozy, S.A., 1992. Insulin-stimulated vascular relaxation: role of Ca^{2+}-ATPase. Am. J. Hypertens. 5, 637–641.