J. Pharm. Pharmacol. 1998, 50: 1117–1120
Received March 13, 1998
Accepted June 4, 1998

Pharmacological Modulation of Endothelial Function by Insulin in the Rat Aorta

D. W. LAIGHT, A. V. KAW, M. J. CARRIER AND E. E. ÄNGGÅRD

The William Harvey Research Institute, St Bartholomew's & the Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK

Abstract

Nitric oxide (NO)-mediated vasodilation induced by hyperinsulinaemia might involve an indirect action which promotes agonist-stimulated endothelial function. Our aim was to attempt to demonstrate such modulation of endothelium-dependent vasodilation by insulin in the rat isolated aorta.

We found that vasodilation in response to acetylcholine, but not to adenosine diphosphate (ADP), histamine or the calcium ionophore A23187, was modestly enhanced after 20-min pretreatment with human insulin (100 nM) whereas endothelium-independent responses to the NO donor sodium nitroprusside were not significantly affected.

Human insulin thus has the acute pharmacological action of selectively enhancing muscarinic receptor-mediated endothelial function in rat aortic vascular smooth muscle in-vitro.

Studies by Steinberg et al (1994) and Scherrer et al (1994) in man have indicated that local acute hyperinsulinaemia elicits nitric oxide (NO)-mediated (Furchgott & Zawadzki 1980; Palmer et al 1987) vasodilation in skeletal muscle. This implies the participation of endogenous insulin in the postprandial, endothelium-dependent vasodilation implicated in the delivery of blood-borne glucose to insulin-sensitive sites (Laakso et al 1992; Baron 1994, 1996). It has further been suggested that insulin-elicited vasodilation might be indirect via enhancement of agonist-stimulated endothelial function (Taddei et al 1995; Taddei & Salvetti 1997).

The rat isolated aorta is a convenient and well-researched vascular preparation which affords a simple experimental in-vitro system with which to investigate the vasoactivity of insulin. Rat aortic smooth muscle has been widely used to demonstrate endothelium-dependent modulation of contractile reactivity by insulin peptide (Karasu & Altan 1993; Peuler et al 1993; Standley et al 1993; Wu et al 1994; Han et al 1995; Lembo et al 1995) and Gros et al (1994) documented endothelium-dependent augmentation of vasorelaxation to isoprenaline by insulin in the same preparation (although the precise relevance of the endothelium in this action is not clear because isoprenaline can act directly on vascular smooth muscle (Graves & Poston 1993)).

Our aim in this study was to increase our understanding of insulin's proposed modulation of endothelial function in the rat isolated aorta by evaluating the effect of this hormone on vasorelaxation to a number of established endothelium- and NO-dependent agonists.

Correspondence: D. W. Laight, The William Harvey Research Institute, St Bartholomew's & the Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, UK.

Materials and Methods

Materials

N^G-Nitro-L-arginine methyl ester, (−)-noradrenaline bitartrate, acetylcholine hydrochloride, adenosine diphosphate, histamine dihydrochloride, isoprenaline, sodium nitroprusside and phenylephrine were obtained from Sigma.

Procedure

Male Wistar rats, 250–300 g, were anaesthetized with sodium pentobarbital (60 mg kg^{-1} i.p.) and killed by cervical dislocation. The thoracic aorta was carefully excised and cut into rings approximately 2 mm in length. Rings prepared from proximal and distal aorta were mounted at random under a resting tension of 2 g in organ baths in physiological salt solution (composition, mM: NaCl 133; KCl 4·7; NaH_2PO_4 1·35; $NaHCO_3$ 16·3; $MgSO_4$ 0·61; $CaCl_2$ 2·52; D-glucose, 7·8) gassed

with carbogen and warmed to 37°C. Responses of ring preparations were measured by means of Grass FT03C force transducers and recorded on a Grass model 79 polygraph.

Rings were left to stabilize for 1 h during which time the physiological salt solution was changed every 15 min. Rings were then exposed at random either to human insulin (100 nM) or to its vehicle (0·1% w/v bovine serum albumin) for 20 min before precontraction with a concentration of noradrenaline which elicited approximately 90% of the maximum response (100 nM). Insulin (100 nM) or its vehicle was present throughout the experiment. Endothelium-dependent vasorelaxation to acetylcholine (1 nM–1 μM), ADP (0·03–10 μM), histamine (0·1–300 μM) and the calcium ionophore A23187 (3–100 nM), and endothelium-independent vasorelaxation to sodium nitroprusside (1–300 nM) was then assessed in separate preparations.

Statistics

Data are expressed as means ± s.e.m. The significance of differences between two means was evaluated by means of Student's two-tailed *t*-test which was paired for observations made on matched ring preparations. Values of pD_2 and AUC (area under the plasma-concentration–time curve) were determined by use of Prism (GraphPad Software, USA).

Results

Effects of insulin on endothelium-dependent vasodilation

Pretreatment with insulin (100 nM) for 20 min elicited a significant, 1·5-fold leftward shift in vasodilation to acetylcholine (Figure 1 and Table 1). A shorter (10 min) pretreatment with insulin (100 nM, n = 6) was without significant effect whereas a longer (30 min) pretreatment (n = 6) was determined to be no more effective than 20-min pretreatment (data not shown). Pretreatment for 20 min with a lower concentration of insulin (10 nM, n = 5) was without significant effect (data not shown). In contrast with vasorelaxation to acetylcholine, that to ADP, histamine, A23187, and endothelium-independent vasorelaxation to sodium nitroprusside, were not significantly affected by 20-min pretreatment with insulin (100 nM) (Figure 2 and Table 1). Precontraction to noradrenaline (100 nM) was modestly depressed, from 1·53 ± 0·06

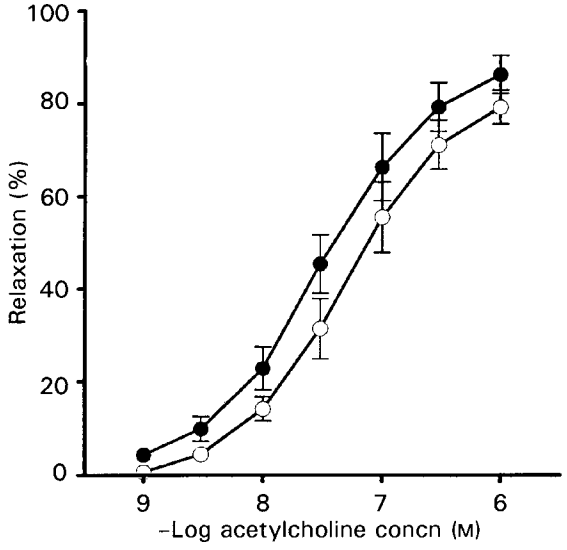

Figure 1. Vasorelaxation to acetylcholine in the rat isolated aorta: effect of 20-min pretreatment with vehicle (○) or human insulin (100 nM; ●). Values are means ± s.e.m. (n = 8).

Table 1. Vasodilation in the rat isolated aorta in response to agonists—effect of 20-min pretreatment with human insulin (100 nM).

Agonist	n	AUC	pD_2	E_{max} (%)
		Vehicle		
Acetylcholine	8	109·1 ± 12·2	7·26 ± 0·13	79·2 ± 3·7
Adenosine diphosphate	6	61·4 ± 9·2	5·94 ± 0·08	56·8 ± 5·6
Histamine	5	176·8 ± 11·9	5·50 ± 0·13	91·1 ± 2·4
A23187	6	68·7 ± 4·0	N.D.†	86·6 ± 2·1
Sodium nitroprusside	6	162·4 ± 15·6	7·68 ± 0·18	100 ± 0
Isoprenaline	6	256·1 ± 16·6	6·48 ± 0·21	90·9 ± 1·4
		Insulin		
Acetylcholine	8	135·3 ± 13·0*	7·45 ± 0·21**	86·4 ± 4·1*
Adenosine diphosphate	6	55·9 ± 8·8	5·99 ± 0·10	50·6 ± 6·1
Histamine	5	165·5 ± 16·7	5·47 ± 0·16	88·4 ± 3·5
A23187	6	68·8 ± 3·9	N.D.	88 ± 2·1
Sodium nitroprusside	6	178·2 ± 9·6	7·79 ± 0·11	100 ± 0
Isoprenaline	6	235·5 ± 15·6*	6·12 ± 0·21**	92·4 ± 1·0

Values are means ± s.e.m. AUC, area under the plasma-concentration–time curve; pD_2, negative logarithm to base 10 of concentration of agonist eliciting 50% of the maximum response; Emax, maximum response. * $P < 0·05$, ** $P < 0·01$, significantly different from result for vehicle. N.D.,‡ not determinable.

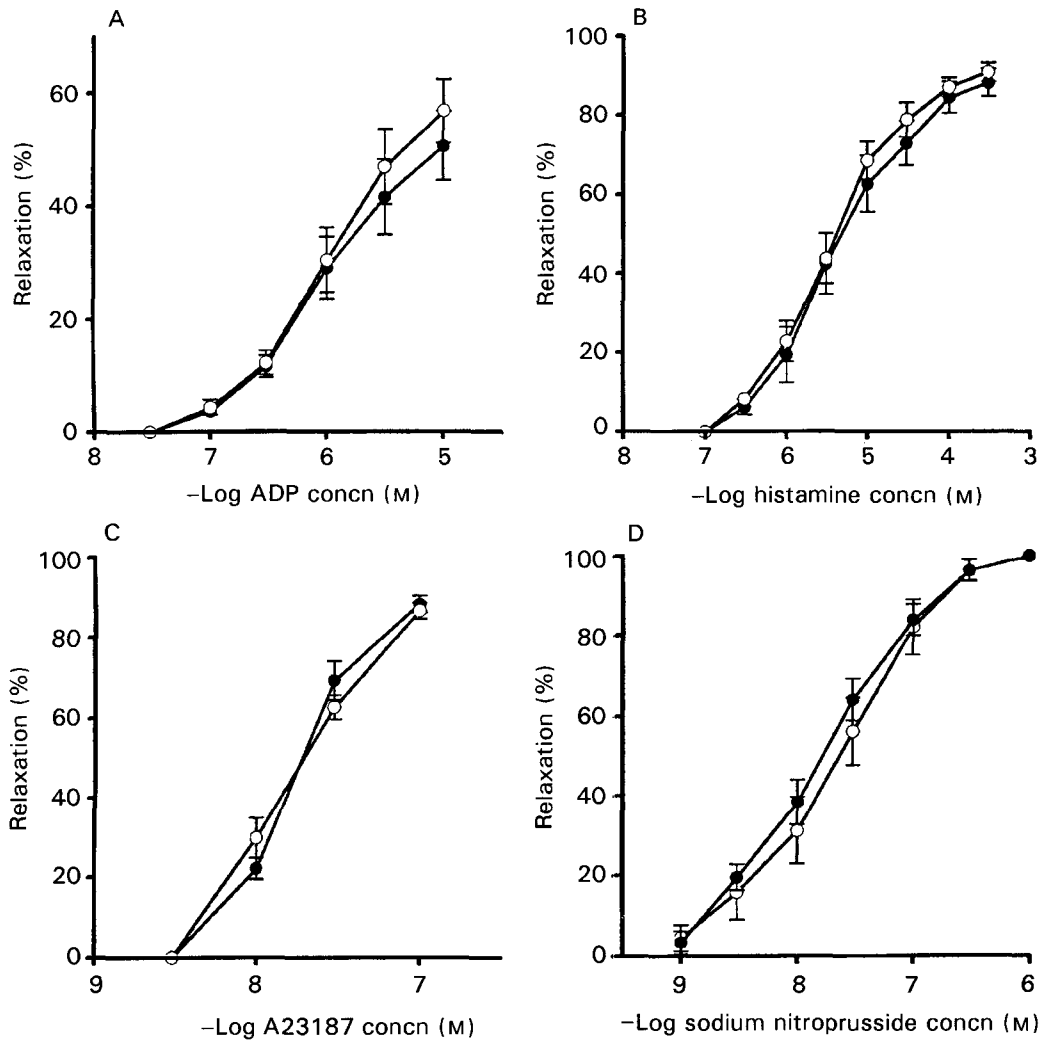

Figure 2. Vasorelaxation to: A. ADP (n = 6); B. histamine (n = 5); C. A23187 (n = 6) and D. sodium nitroprusside (n = 6) in the rat isolated aorta: effect of 20-min pretreatment with vehicle (○) or human insulin (100 nM; ●). Values are means ± s.e.m.

to 1·41 ± 0·10 g ($P < 0·01$, n = 31) by 20-min pretreatment with insulin (100 nM).

Discussion

Endothelium-dependent vasorelaxation to acetylcholine in the rat isolated aorta was found to be modestly enhanced by pharmacological concentrations of insulin whereas vasorelaxation to an NO donor was not significantly affected. These results are therefore consistent with studies in resistance vessels of skeletal muscle in man (Scherrer et al 1994; Steinberg et al 1994; Taddei et al 1995) which exclude a non-specific effect of insulin in the augmentation of vasodilator reactivity to NO and point instead to an effect on NO biosynthesis or release, or both.

Because in this study insulin did not modify endothelium-dependent responses to ADP, histamine or the receptor-independent agonist A23187, it is likely that insulin acts selectively on the muscarinic receptor pathway leading to the stimulation of constitutive, endothelial NO synthase. This notion is supported by a recent report (Wan et al 1996) that tyrosine kinase activity, a critical component of the insulin-stimulated signal cascade (White & Khan 1994), has the potential to influence muscarinic receptor signalling in cultured cells. Our finding is potentially very significant given that the chief evidence in man of enhancement in endothelium-dependent vasodilation as a result of hyperinsulinaemia has been obtained by use of acetylcholine or methacholine (Steinberg et al 1994; Taddei et al 1995).

The concentrations of insulin reported in the literature to be vasoactive in-vitro are frequently outside the normal physiological range, which could reflect a decrease in tissue responsiveness to insulin outside the normal in-vivo metabolic milieu (Muggeo et al 1977; Bar et al 1979). The choice and site of

vessel for in-vitro study might also be a factor given a conceivable heterogeneity in the density of vascular insulin receptors (Muggeo et al 1977; Bar et al 1979; Baron & Brechtel 1993) and small arterial vessels regulating resistance in insulin-sensitive tissue such as skeletal muscle and adipose tissue seem to be particularly responsive to the vasodilator effect of insulin (McNally et al 1995; Chen & Messina 1996; Walker et al 1997). It is also feasible that some of the vasoactive effects reported for insulin in-vitro might be mediated by the insulin-like growth factor receptor system for which insulin has a relatively low potency (Bar et al 1988; Haylor et al 1991; Walsh et al 1996; Zeng & Quon 1996).

In conclusion, the acute pharmacological action of human insulin of selectively enhancing vasodilation to acetylcholine can be demonstrated in rat aortic smooth muscle. Although our findings in rat conductance tissue in-vitro agree qualitatively with the vasoactivity of insulin reported in man, studies in resistance vessels of insulin-sensitive tissue in-situ or in-vivo are likely to result in more physiological investigations of the vascular actions of insulin.

Acknowledgement

This work was supported by Lipha s.a., Lyon, France.

References

Bar, R. S., Harrison, L. C., Muggeo, M., Gorden, P., Kahn, C. R., Roth, J. (1979) Regulation of insulin receptors in normal and abnormal physiology in humans. Adv. Intern. Med. 24: 23–52

Bar, R. S., Boes, M., Dake, B. L., Booth, B. A., Henley, S. A., Sandra, A. (1988) Insulin, insulin-like growth factors, and vascular endothelium. Am. J. Med. 85: 59–70

Baron, A. D. (1994) Haemodynamic actions of insulin. Am. J. Physiol. 267: E186–E202

Baron, A. D. (1996) The coupling of glucose metabolism and perfusion in human skeletal muscle. The potential role of endothelium-derived nitric oxide. Diabetes 45: S105–S109

Baron, A. D., Brechtel, G. (1993) Insulin differentially regulates systemic and skeletal muscle vascular resistance. Am. J. Physiol. Endocrinol. Metab. 265: E61–E67

Chen, Y. L., Messina, E. J. (1996) Dilation of isolated skeletal muscle arterioles by insulin is endothelium dependent and nitric oxide mediated. Am. J. Physiol. 39: H2120–H2124

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373–376

Graves, J., Poston, L. (1993) β-Adrenoceptor agonist-mediated relaxation of rat isolated resistance arteries: a role for the endothelium and nitric oxide. Br. J. Pharmacol. 108: 631–637

Gros, R., Borkowski, K. R., Feldman, R. D. (1994) Human insulin-mediated enhancement of vascular beta-adrenergic responsiveness. Hypertension 23: 551–555

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

Haylor, J. H., Singh, I., El Nahas, A. M. (1991) Nitric oxide synthesis inhibitor prevents vasodilation by insulin-like growth factor 1. Kidney Int. 39: 333–335

Karasu, C., Altan, V. M. (1993) The role of endothelial cells on the alterations in vascular reactivity induced by insulin-dependent diabetes mellitus: effects of insulin treatment. Gen. Pharmacol. 24: 743–755

Laakso, M., Edelman, G., Brechtel, G., Brown, A. D. (1992) Effects of epinephrine on insulin-mediated glucose uptake in whole body and leg muscle in humans: role of blood flow. Am. J. Physiol. 263: E199–E204

Lembo, G., Iaccarino, G., Vecchione, C., Rendina, V., Trimarco, B. (1995) Insulin modulation of vascular reactivity is already impaired in prehypertensive spontaneously hypertensive rats. Hypertension 26: 290–293

McNally, P. G., Lawrence, I. G., Watt, P. A. C., Hillier, C., Burden, A. C., Thurston, H. (1995) The effect of insulin on the vascular reactivity of isolated resistance arteries taken from healthy volunteers. Diabetologia 38: 467–473

Muggeo, M., Bar, R. S., Roth, J. (1977) Change in affinity of insulin receptors following oral glucose in normal adults. J. Clin. Endocrinol. Metab. 44: 1206–1209

Palmer, R. M. J., Ferrige, A. G., Moncada, S. (1987) Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524–526

Peuler, J. D., Johnson, B. A., Phare, S. M., Sowers, J. R. (1993) Sex-specific effects of an insulin secretagogue in stroke-prone hypertensive rats. Hypertension 22: 214–220

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

Standley, P. R., Ram, J. L., Sowers, J. R. (1993) Insulin attenuation of vasopressin-induced calcium responses in arterial smooth muscle from Zucker rats. Endocrinology 133: 1693–1699

Steinberg, H. O., Brechtel, G., Johnson, A., Fineberg, N., Baron, A. D. (1994) Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. J. Clin. Invest. 94: 1172–1179

Taddei, S., Salvetti, A. (1997) Insulin and vascular reactivity. Nutr. Metab. Cardiovasc. Dis. 7: 117–123

Taddei, S., Virdis, A., Mattei, P., Natali, A., Ferrannini, E., Salvetti, A. (1995) Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. Circulation 92: 2911–2918

Walker, A. B., Savage, M. W., Dores, J., Williams, G. (1997) Insulin-induced attenuation of noradrenaline-mediated vasoconstriction in resistance arteries from Wistar rats is nitric oxide dependent. Clin. Sci. 92: 147–152

Walsh, M. F., Barazi, M., Pete, G., Muniyappa, R., Dunbar, J. C., Sowers, J. R. (1996) Insulin-like growth factor I diminishes in vivo and in vitro vascular contractility-role of vascular nitric oxide. Endocrinology 137: 1798–1803

Wan, Y., Kurosaki, T., Huang, X.-Y. (1996) Tyrosine kinases in activation of the MAP kinase cascade by G-protein-coupled receptors. Nature 380: 541–544

White, M. F., Kahn, C. R. (1994) The insulin signalling system. J. Biol. Chem. 269: 1–4

Wu, H. Y., Jeng, Y. Y., Yue, C. J., Hsueh, W. A., Chan, T. M. (1994) Endothelial-dependent vascular effects of insulin and insulin-like growth factor I in the perfused rat mesenteric artery and aortic ring. Diabetes 43: 1027–1032

Zeng, G., Quon, J. (1996) Insulin-stimulated production of nitric oxide is inhibited by wortmannin. J. Clin. Invest. 98: 894–898