Insulin-Induced Vasodilatation of Internal Carotid Artery

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The increase in leg and forearm blood flow induced by insulin could be secondary to its metabolic effect on glucose uptake. We therefore investigated whether insulin causes vasodilation of the internal carotid artery, since the brain is not dependent on insulin for glucose uptake, to demonstrate that the vasodilatory effect of insulin is primary and independent of its metabolic effect. Internal carotid artery diameter was continuously monitored using a 7.5-MHz transducer linked to an Acuson XP10 ultrasonograph (Mountainview, CA) during infusion of 125 mL 10% dextrose mixed with 3 U regular insulin and 5 mmol potassium chloride over 1 hour. The internal carotid artery diameter increased progressively with time from a mean of 5.4 ± 1 mm to 5.7 ± 1 mm at 15 minutes, 5.9 ± 1.1 mm at 30 minutes, 6 ± 1.1 mm at 45 minutes, and 6.1 ± 1.1 mm at 60 minutes ($P < .05$), an increase of 13% over baseline. Glucose was maintained between 93 and 106 mg/dL, and insulin increased from 15 ± 14 μU/mL and was maintained between 34 and 47 μU/mL. There was no change in mean arterial blood pressure (MABP) or heart rate during the infusion. We conclude that insulin dilates the internal carotid artery consistently at physiological concentrations, probably independently of glucose uptake by the brain. Alterations in this effect of insulin may be of relevance in the pathogenesis of abnormalities of cerebral blood flow in type 1 and type 2 diabetics as described by our group previously.

SYSTEMIC INFUSION OF INSULIN has been shown to increase blood flow in the forearm and leg.[1-3] We have also demonstrated a vasodilatory effect of insulin in the cephalic vein.[4] These vasodilatory effects of insulin are thought to be mediated by the nitric oxide (NO) synthase–guanylate cyclase pathway.[3,4] There is also a report suggesting that at least part of this effect may be mediated by β-adrenergic mechanisms.[1] While our study on the cephalic vein is based on the direct measurement of venous diameter approximately 1 cm from the site of delivery of insulin and is therefore indicative of a direct effect of insulin on the cephalic vein at the site of infusion, the investigation demonstrating insulin-induced increases in blood flow in the forearm and leg does not define the site of action of insulin, especially since insulin is infused systemically in these experiments. Furthermore, there is the possibility that the increase in blood flow caused by insulin may be secondary to metabolic events occurring in the insulin-responsive end-organ in the forearm and leg, ie, the skeletal muscle. Local metabolic effects may decrease the local vascular tone, which in turn may decrease vascular resistance and thus increase flow.

The brain is not dependent on insulin for its glucose uptake,[5,6] and the internal carotid artery is the major artery supplying the cerebral hemispheres[7]; a demonstration of vasodilatation by insulin in this arterial system would eliminate the confounding variable of insulin's metabolic action. Thus, we have now investigated whether insulin causes vasodilatation of the internal carotid artery to demonstrate that the vasodilatory action of insulin is primary and independent of its metabolic effect. Furthermore, we have previously demonstrated that cerebrovascular reactivity is abnormal in patients with diabetes mellitus,[8,9] a finding that has been confirmed by other groups.[10,11] These studies demonstrate that while basal cerebral blood flow in diabetics is not altered, the response to CO_2 and other cerebrovascular vasodilators is diminished or absent. To date, no satisfactory explanation has been found for these abnormalities. If indeed insulin is a potent cerebral vasodilator, the combination of a lack of insulin and insulin resistance may provide a possible mechanism for the abnormalities in cerebrovascular reactivity in diabetes mellitus.

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SUBJECTS AND METHODS

Subjects

Fifteen healthy subjects (13 males and two females aged 33 ± 10 years; range, 22 to 53) who were nonsmokers and normotensive (blood pressure <140/90 mm Hg) with a fasting blood glucose of 77 ± 8 mg/dL, body mass index 24.7 ± 2.2 kg/m^2, weight 78 ± 5.5 kg, total cholesterol 173 ± 25 mg/dL, and triglycerides 126 ± 68 mg/dL participated in the study. The experimental protocol was approved by the Institutional Review Board on Human Investigation. All subjects provided written informed consent for the investigation.

General Procedure

Subjects were studied in the supine position and were instructed to rest their head on a special pillow designed to minimize neck movement. The antecubital veins were cannulated in both arms with a 21-gauge/23-gauge cannula. One of these was used to infuse the intravenous solutions (normal saline and dextrose solution premixed with insulin and potassium), and the other was used for blood sampling. The blood pressure and heart rate were monitored every 5 minutes throughout the infusion. Three electrodes were placed on the chest for continuous recording of the electrocardiogram.

Ultrasonography

An Acuson 128 XP10 ultrasonograph (Mountainview, CA) with a 7.5-MHz linear-array transducer was used to image the right or left internal carotid artery. The transducer was held at a constant distance from the skin (to avoid local pressure effects) and at a fixed point over the internal carotid artery with a stand that was specially designed. An interface of ultrasound jelly warmed to room temperature was used between the transducer and the skin. On a super VHS videocassette, a two-dimensional and an M-mode image of the cross-section of the internal carotid artery was recorded. The M-mode image with the cursor placed at the center of the cross-sectional image was used for measurement of the diameter (Fig 1).

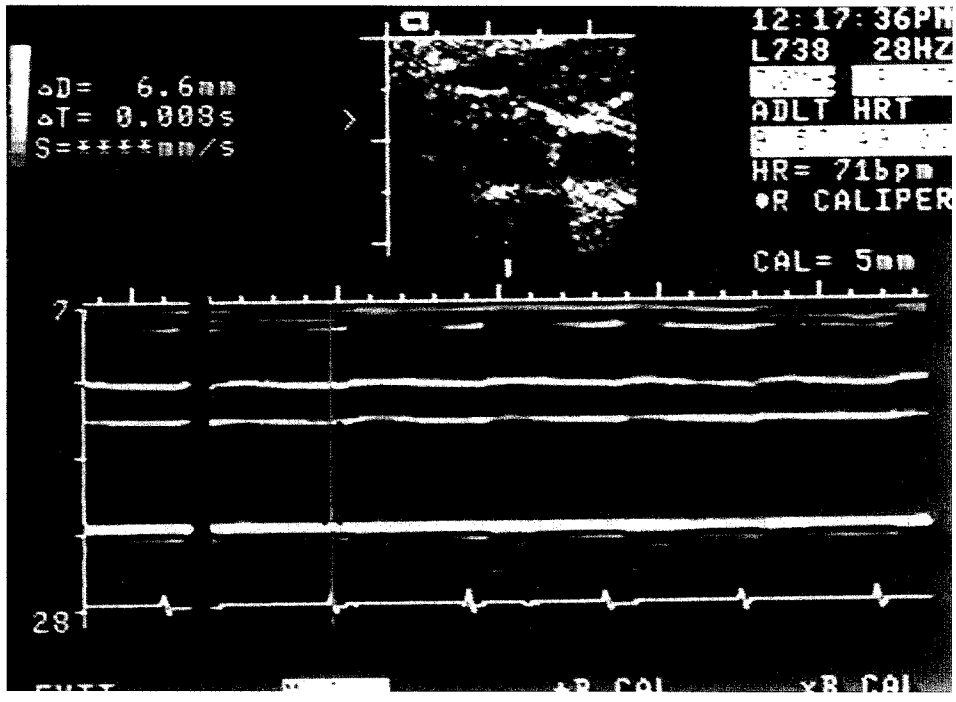

Fig 1. Split-screen image of the internal carotid artery. The cursor is placed at the center of the 2-dimensional image while measurements are made on the M-mode image at the height of the R wave. Each line on the scale for the M-mode image is 5 mm.

The diameters were measured at the height of the R wave, and at each time point measurements were taken for 10 cardiac cycles by two observers, one of whom was blinded to the experiment. The average of 10 readings was taken as the representative diameter of the internal carotid artery for that time point. The maximal interobserver and intraobserver variability was 0.1 mm for this technique. Thus, the interobserver and intraobserver coefficient of variation was 2% for arteries of 5.0-mm diameter.

Experimental Protocol

The baseline diameter of the internal carotid artery was recorded after the subjects rested comfortably for 15 minutes. During this period, normal saline was infused at 125 mL/h. Once the baseline was obtained, the normal saline infusion was stopped and 125 mL 10% dextrose premixed with 3 U soluble insulin and 5 mmol potassium chloride was infused into an antecubital vein over 1 hour. An infusion pump (Lifecare Pump model 4P; Abbott, North Chicago, IL) was used to infuse both solutions. The internal carotid artery diameter was recorded at 0, 15, 30, 45, and 60 minutes during the dextrose infusion, and a final recording was made 15 minutes after the end of the infusion. Blood samples were taken at baseline and at 15, 30, 45, and 60 minutes for measurement of plasma glucose and immunoreactive insulin.

Analytical Methods

Blood samples were collected in EDTA plasma and serum separation tubes for estimation of insulin and glucose. The samples were centrifuged at 2,800 rpm for 10 minutes at room temperature. Plasma and serum samples were stored at −70°C. Insulin was measured using a radioimmunoassay kit from Linco Research Laboratories (St Louis, MO). The assay is sensitive for insulin concentrations between 2 and 200 μU/mL. The interassay and interassay coefficient of variation is less than 5%. Glucose was measured immediately using a Hemocue Ängelholm, Sweden) B-glucose photometer. In this method, the colored compound that is read by the photometer is produced using a modified glucose dehydrogenase method that catalyzes the reaction that produces NADH from glucose. The NADH reacts with a chromogen compound, methyl thiazolyl diphenyl tetrazolium (MTT), to form formazan, which is quantified photometrically using a two-wavelength photometric method at 660 to 840 nm. This method is linear between blood glucose concentrations of 36 and 400 mg/dL.

Agents

Regular insulin was used (Humulin R, recombinant DNA origin, Eli Lilly & Co, Indianapolis, IN). The potassium chloride was from Abbott. Normal saline and 10% dextrose were from Baxter Healthcare (Deerfield, IL).

Statistical Analysis

All diameters are expressed in millimeters as the mean ± SD. The statistical significance of the change with time was determined by one-way repeated-measures ANOVA. Multiple comparisons between time points were made using the Student-Newman-Keuls method. A P value less than .05 was taken as significant for all tests. The Sigmastat statistics package (Jande Scientific, San Rafael, CA) was used to analyze all data.

RESULTS

Diameter of the Internal Carotid Artery

Insulin caused a significant increase in the diameter of the internal carotid artery. The diameter increased from a mean of 5.4 ± 1 mm to 5.7 ± 1 mm at 15 minutes, 5.9 ± 1.1 mm at 30 minutes, 6 ± 1.1 mm at 45 minutes, and 6.1 ± 1.1 mm at 60 minutes. The diameter returned to the baseline within 15 minutes of stopping the infusion in all subjects (Fig 2). The diameter was significantly greater than the baseline value at all time points and increased significantly with time, except between 30 and 45 minutes ($P < .05$). When expressed as a percentage of baseline, the internal carotid artery diameter increased significantly from 100% to 104% ± 5% at 15 minutes, 108% ± 5% at 30 minutes, 110% ± 7% at 45 minutes, and 113% ± 7% at 60 minutes ($P < .05$). There was no change

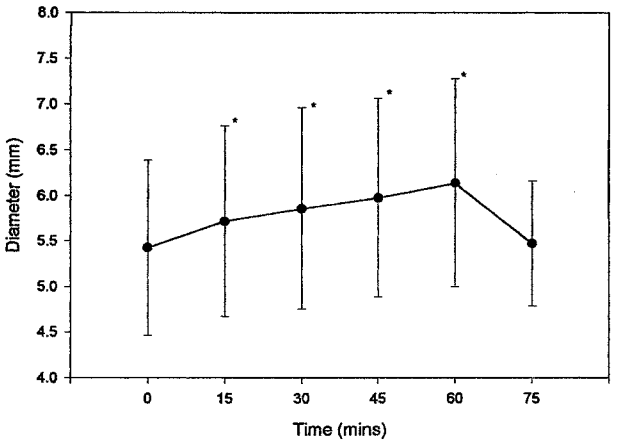

Fig 2. Change of internal carotid artery diameter with time during infusion of dextrose, insulin, and potassium solution. There was no change in the baseline diameter during infusion of normal saline. Data are the mean ± SD. *$P < .05$ *v* baseline diameter (1-way repeated-measures ANOVA).

in the baseline diameter during the initial 15-minute period of infusion with normal saline.

Glucose

Glucose levels were maintained between 93 and 106 mg/dL throughout the infusion (Table 1).

Insulin

Immunoreactive insulin increased from a mean of 15 ± 14 μU/mL and was maintained between 34 and 47 μU/mL throughout the infusion (Table 1).

Mean Arterial Blood Pressure

There was no change in the mean arterial blood pressure (MABP) during the infusion. It was 89 ± 5 mm Hg at baseline and 91 ± 11 mm Hg at the end of the infusion ($P = .34$; Table 1).

Heart Rate

The heart rate varied between 65 and 71 beats per minute throughout the infusion (nonsignificant).

DISCUSSION

Our data show that insulin causes a significant increase in the diameter of the internal carotid artery. The vasodilatory effect is significant at 15 minutes and increases progressively with time.

Table 1. Glucose, Insulin, and MABP at Different Time Points

Variable	Baseline	15 Minutes	30 Minutes	45 Minutes	60 Minutes
Glucose (mg/dL)	105 ± 13	110 ± 15	101 ± 15	96 ± 21	93 ± 23
Insulin (μU/mL)	15 ± 14	40 ± 22	47 ± 25	34 ± 10	35 ± 10
MABP (mm Hg)	89 ± 5	88 ± 6	89 ± 8	91 ± 9	91 ± 11

NOTE. Data are the mean ± SD analyzed by 1-way repeated-measures ANOVA (P = nonsignificant).

The arterial diameter reverts to baseline 15 minutes after cessation of the infusion. The magnitude of the increase in diameter is 13% at 60 minutes. The vasodilatation occurs in the absence of hypoglycemia and a significant change in the MABP and heart rate, and is evident at physiological concentrations of insulin between 34 and 47 μU/mL.

The internal carotid artery, which supplies the major portion of the cerebral cortex and upper brainstem, is thus subject to the vasodilatory effect of insulin, although the brain is not dependent on insulin for its glucose uptake. Thus, the vasodilatory effect of insulin in this setting is primary and independent of any effect of insulin on glucose metabolism in the brain. These findings are consistent with a recent study showing that insulin caused dilation of isolated arteries from skeletal muscle.[12] Furthermore, the vasodilatory effect of insulin on the extracranial internal carotid artery was demonstrated well away from the brain and the extracranial supply of the internal carotid artery is limited to a small portion of the forehead and nose that is also supplied by branches of the facial artery[7]; it is unlikely that this effect is dependent on local tissue factors in the brain or the metabolic effects on the extracranial structures supplied. It has been shown that the vasodilatory effect of insulin is mediated through the NO–guanylate cyclase (NO-cGMP) pathway.[3,4] At the cellular level, it has been shown that insulin induces the acute release of NO by endothelial cells in culture.[13] In addition, we have recently demonstrated that insulin induces the increased expression of NO synthase in human aortic endothelial cells in vitro.[14] These in vivo and cellular effects may help to explain the vasodilatory effects of this hormone.

The fact that insulin induces carotid vasodilatation may have implications for the defects in cerebral blood flow that we have previously demonstrated in diabetics. The presence of insulin resistance in patients with type 2 diabetes mellitus[15] and the lack of insulin in insulin-dependent diabetes mellitus may affect the bioavailability of NO from the endothelium and thus the endothelium-dependent vasodilatation, since it has been demonstrated that insulin-induced vasodilatation is NO-cGMP–dependent.[3,4] This may also explain the impairment of the cerebral blood flow response to CO_2 inhalation, an effect now known to be mediated by NO,[16] that has been shown in diabetes,[8,9] although both neuronal and endothelial NO may be responsible for the cerebral vasodilatory effect of CO_2 and there is as yet no evidence of impaired neuronal NO bioavailability in diabetes.[17] It has been demonstrated recently that if euglycemia is maintained, cerebral blood flow or glucose uptake are not affected by physiological insulin concentrations in diabetic men.[18] We are currently investigating whether patients with non–insulin-dependent diabetes mellitus (NIDDM) and/or obesity have impaired carotid vasodilatory responses to insulin. It has been previously demonstrated that NIDDM and obesity are associated with impaired increments in leg blood flow following insulin.[19,20] We have also shown that insulin-induced vasodilatation in the cephalic vein is impaired/absent in obese and NIDDM[21] subjects.

Early recanalization and good collateral blood supply have been shown to have a favorable impact on infarct size and

outcome in subjects with middle cerebral artery occlusion.[22,23] One study has correlated the presence and adequacy of the collateral blood supply with asymptomatic internal carotid artery occlusion.[24] Insulin has been shown to reduce ischemic brain damage in various animal models.[25,26] The blood glucose–lowering effect of the hormone[27] and a neuroprotective effect mediated via a growth factor mechanism[28] have been offered as likely explanations for this effect. Nitroprusside, an endothelial-independent NO donor, has been shown to improve blood flow and decrease brain damage in rats after focal cerebral ischemia.[29] Therefore, it is possible that the vasodilatory effect of insulin may improve the collateral blood flow and may thus be responsible for its beneficial effect on ischemic cerebral damage. Apart from the maintenance of normoglycemia,[30] this acute vasodilatory effect might thus have additional therapeutic implications in the management of acute stroke.

We conclude that at physiological concentrations, insulin induces a consistent 10% to 15% vasodilation in the internal carotid artery, probably independent of its effect on glucose uptake. This effect of insulin on a major cerebral artery probably has important implications for the abnormalities in cerebral vascular reactivity known to occur in diabetics and an absence or resistance to this action of insulin may predispose or worsen the prognosis of cerebrovascular disease in diabetics. The acute vasodilatory effects might have therapeutic implications in the management of acute stroke.

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