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PRELIMINARY REPORT

Insulin-Induced Vasodilation Is Dependent on Tetrahydrobiopterin Synthesis

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Insulin has been shown to elicit vasodilation through increases in nitric oxide (NO) production. To examine whether insulin may modulate the availability of tetrahydrobiopterin (BH_4) (an absolute cofactor requirement for NO synthase activation), we studied the effects of insulin (150 nmol/L) on femoral arterial reactivity (to norepinephrine [NE]) in the presence and absence of 2,4-diamino-6-hydroxypyrimidine (DAHP), a specific inhibitor of BH_4 production. Our data indicate that inhibition of BH_4 synthesis results in an attenuation in the vasodepressor effect of insulin. One possibility is that insulin may regulate NO production by increasing cofactor (BH_4) availability for activation of NO synthase.

ONE OF THE HIGHLIGHTS OF endocrine research has been the observation that insulin, in addition to its well-known effects on carbohydrate, protein, and fat metabolism, exerts vasodilator effects. Insulin has specific and physiologically relevant effects to increase skeletal muscle blood flow. In recent years, results have repeatedly shown that intravenous insulin, independent of glucose changes, increases blood flow in the leg.[1-3] By combining the euglycemic clamp with the leg-balance technique, these investigators also demonstrated that insulin-induced vasodilation was specific for skeletal muscle. This effect was dose-dependent and occurred at physiological insulin concentrations with an apparent ED_{50} of 35 to 40 μU/mL in lean insulin-sensitive subjects.[4] The vasodilating action of insulin has been confirmed by several groups over a range of physiological insulin concentrations and by using different techniques.[5-10]

Much current attention has focused on the interaction between insulin and the endothelium-derived nitric oxide (NO) system in mediating vasodilation. There is now compelling evidence that insulin-mediated vasodilation in humans is NO-dependent. Studies by Steinberg et al[11] have provided evidence for this mechanism. In their studies, intrafemoral artery infusion of the specific inhibitor of endothelium-derived NO synthesis, N^G-monomethyl-L-arginine (L-NMMA) were performed under basal conditions in healthy volunteers and leg blood flow was measured by thermodilution. In a separate group, L-NMMA infusions were performed after 3 hours of hyperinsulinemia during a euglycemic clamp designed to increase leg blood flow approximately twofold. At baseline, L-NMMA caused an approximately 25% decrease in leg blood flow. During hyperinsulinemia, leg blood flow increased approximately twofold, and in contrast to baseline, L-NMMA caused an approximately 50% decrease in leg blood flow, indicating that insulin-mediated vasodilation was NO-dependent. Additional studies have also indicated that blockade of NO synthesis (with L-NMMA) abrogates insulin-mediated vasodilation.[1,11] Although the exact mechanism(s) through which insulin interacts with the NO pathway in humans is unclear, studies indicate that this may involve synthesis/release of NO, but not NO action on vascular smooth muscle cells (VSMC).[1,11]

In the endothelial cell, NO is synthesized from L-arginine by a constitutive NO synthase (cNOS).[16] The activity of cNOS is strictly dependent on tetrahydrobiopterin (BH_4), a cofactor for cNOS activation.[12,13] Given the central role of BH_4 in endothelial NO production, we hypothesized that insulin-mediated

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vasodilation may be mediated through increasing availability/synthesis of BH_4 and thereby increasing NO production. To examine this proposition, we studied the effects of insulin on femoral arterial tone in presence and absence of 2,4-diamino-6-hydroxypyrimidine (DAHP), a selective guanosine triphosphate (GTP) cyclohydrolase I inhibitor that blocks BH_4 synthesis (Fig 1B).

MATERIALS AND METHODS

Femoral arteries from male Sprague-Dawley rats (n = 10) were dissected, cleaned of adherent connective tissue, and cut into rings. For each rat, two rings from the femoral artery with intact endothelium were used. The tissues were suspended on wire hooks in isolated tissue baths containing modified Krebs-Ringer bicarbonate solution as described previously.[14] Each femoral artery was placed under a resting tension of 0.5 g to allow for maximum force generation. After an initial 90-minute equilibration period, the tissues were stimulated according to the following protocol: (1) a cumulative dose-response curve (DRC) to norepinephrine (NE) 10^{-9} to 10^{-5} mol/L, (2) a cumulative DRC to NE 10^{-9} to 10^{-5} mol/L in the presence of insulin (150 nmol/L for 45 minutes), and (3) a cumulative DRC to NE 10^{-9} to 10^{-5} mol/L in the presence of DAHP (2×10^{-3} mol/L for 60 minutes) and insulin. Presence of a functional endothelium was confirmed by relaxation to 10^{-6} mol/L acetylcholine. The concentrations of insulin and DAHP were based on previous in vitro reports.[13,15] To ensure that DAHP itself did not affect vascular responses to NE, control experiments including cumulative DRCs to NE 10^{-9} to 10^{-5} mol/L in the absence and presence of DAHP alone (2×10^{-3} mol/L for 60 minutes) were performed in femoral arteries from male Sprague-Dawley rats in a separate study. Contractile responses to NE were expressed as percentage of maximum contraction in the absence of insulin and DAHP. In addition, agonist pD_2 ($-\log ED_{50}$) values were calculated by nonlinear regression analysis of the DRCs and were used as an index of sensitivity. Results were compared using a repeated-measures ANOVA followed by a Newman Keuls test. A probability of less than .05 was used to indicate significant differences between means.

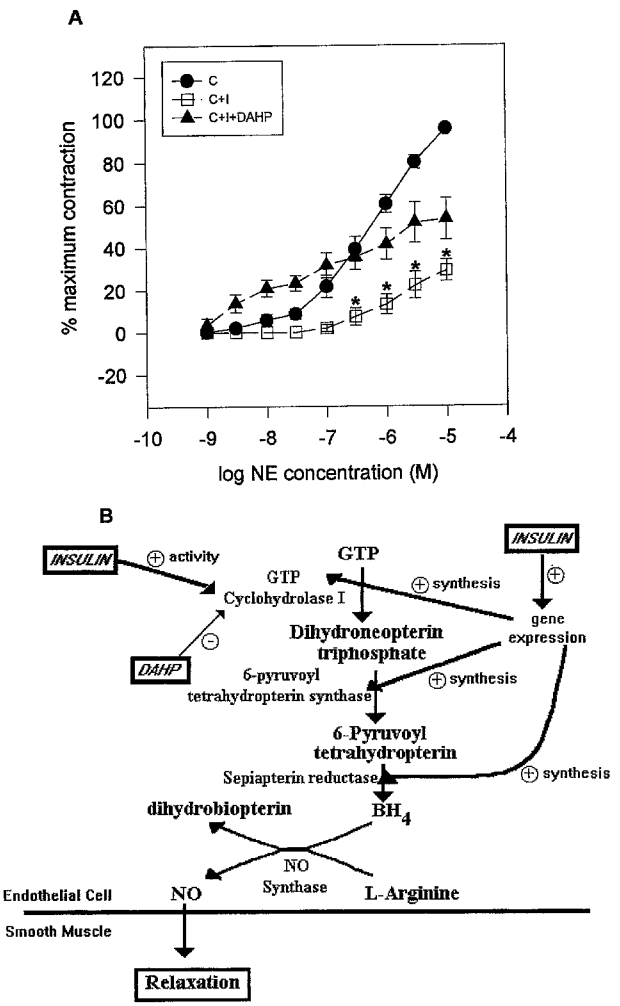

Fig 1. (A) NE DRC of femoral arteries in control Sprague-Dawley rats (n = 10) in the absence (C) and presence of insulin (150 nmol/L for 45 minutes) (C + I) and presence of insulin and DAHP (2×10^{-3} mol/L for 60 minutes) (C + I + DAHP). Each point is presented as the mean ± SEM. *$P < .05$, different from C and C + I + DAHP. (B) Relationship between BH_4 and NO production via NOS in endothelial cells. Schematic depicts the site of action of DAHP and the potential modulatory sites of insulin.

RESULTS AND DISCUSSION

As depicted in Fig 1A, insulin caused marked vasodepressor effects in control femoral arteries (percent maximum contraction 10^{-6} mol/L [NE], control 60.7 ± 4.2 *v* control + insulin 13.2 ± 4.8, $P < .05$). Analysis of the pD_2 values indicated that insulin significantly reduced the sensitivity of femoral arteries to NE (pD_2 values, control 5.98 ± 0.10 *v* control + insulin 3.94 ± 0.17, $P < .05$). The novel observation from this study relates to the effects of DAHP on insulin-mediated attenuation of NE contraction (Fig 1A). In the presence of DAHP, insulin's effects were significantly attenuated (percent maximum contraction 10^{-6} mol/L [NE], control + DAHP + insulin 41.69 ± 7.43 *v* control + insulin, $P > .05$). Furthermore, DAHP prevented insulin-induced attenuation of NE sensitivity (pD_2 values: control + DAHP + insulin 4.59 ± 0.28 *v* control + insulin, $P > .05$). Results from the control experiments showed that DAHP did not affect basal arterial tone (data not shown) or vascular reactivity of femoral arteries to NE (percent maximum contraction 10^{-6} mol/L [NE], without DAHP 74.5 ± 5.0 *v* with DAHP 72.0 ± 11.1).

The data from this study support previous studies demonstrating the role of NO production in mediating insulin's vascular effects[11] and demonstrate for the first time that insulin-mediated vasodilation may be dependent on BH_4 synthesis in rat femoral arteries. Although the exact mechanism through which insulin increases endothelial NO production is not known, this may be related to the effect of insulin on BH_4 availability. This notion is supported by previous studies in rat adrenal glands in which insulin increased the activity of GTP cyclohydrolase I, the first and rate-limiting enzyme responsible for the endogenous biosynthesis of BH_4.[17] Since insulin is known to enhance gene expression of various enzymes, insulin may affect BH_4 availability by increasing the gene expression of GTP cyclohydrolase I or other enzymes involved in the pathway of BH_4 synthesis. Additional support for an interaction between insulin and BH_4 has been shown in a previous study demonstrating a decreased BH_4 level in brains from hypoinsulinemic streptozotocin-induced diabetic rats.[18] Despite the aforementioned postulations, it is important to note that this study does not establish a

causal interaction between insulin and BH_4, but suggests that insulin causes vasodilation through the L-arginine NO pathway and that BH_4 may be a potential modulatory site for insulin-induced vasodilation.

The observation that insulin causes vasodilation has led to the hypothesis that in states of insulin resistance, the effects of insulin on vascular tone may also be blunted. Although the exact contribution of insulin-mediated vasodilation to overall hemodynamics is unclear, studies in insulin-resistant states of obesity, hypertension, and non–insulin-dependent diabetes mellitus demonstrate a blunted effect of insulin on vascular tone.[1-3] Thus, it is possible that resistance to the vasodilator effects of insulin may be important in the development of hypertension in states of insulin resistance.

A question that must be addressed relates to the relative contribution of insulin-mediated vasodilation towards insulin's effects on glucose uptake. Elegant studies by Baron et al have demonstrated that up to 40% of insulin-mediated glucose uptake can be accounted for by insulin-mediated vasodilation.[3] Thus, the lack of vasodilation in insulin-resistant conditions may play a central role in the reinforcement of the insulin-resistant state per se.

In summary, data from this preliminary study support the notion that insulin-mediated vasodilation in rat femoral arteries is mediated through the L-arginine NO pathway and that BH_4 represents a potential site of insulin action. Further studies aimed at measuring BH_4 in arteries in response to insulin are warranted and may prove useful.

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