Acute Vasoconstriction-Induced Insulin Resistance in Rat Muscle In Vivo

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Insulin-mediated changes in blood flow are associated with altered blood flow distribution and increased capillary recruitment in skeletal muscle. Studies in perfused rat hindlimb have shown that muscle metabolism can be regulated by vasoactive agents that control blood flow distribution within the hindlimb. In the present study, the effects of a vasoconstrictive agent that has no direct effect on skeletal muscle metabolism but that alters perfusion distribution in rat hindlimb was investigated in vivo to determine its effects on insulin-mediated vascular action and glucose uptake. We measured the effects of α-methylserotonin (α-met5HT) on mean arterial blood pressure, heart rate, femoral blood flow, hindlimb vascular resistance, and glucose uptake in control and euglycemic insulin-clamped (10 mU · min^{-1} · kg^{-1}) anesthetized rats. Blood flow distribution within the hindlimb muscles was assessed by measuring the metabolism of 1-methylxanthine (1-MX), an exogenously added substrate for capillary xanthine oxidase. α-Met5HT (20 μg · min^{-1} · kg^{-1}) infusion alone increased mean arterial blood pressure by 25% and increased hindlimb vascular resistance but caused no change in femoral blood flow. These changes were associated with decreased hindlimb 1-MX metabolism indicating less capillary flow. Insulin infusion caused decreased hindlimb vascular resistance that was associated with increased femoral blood flow and 1-MX metabolism. Treatment with α-met5HT infusion commenced before insulin infusion prevented the increase in femoral blood flow and inhibited the stimulation of 1-MX metabolism. α-Met5HT infusion had no effect on hindlimb glucose uptake but markedly inhibited the insulin stimulation of glucose uptake ($P < 0.05$) and was associated with decreased glucose infusion rates to maintain euglycemia ($P < 0.05$). A significant correlation ($P < 0.05$) was observed between 1-MX metabolism and hindlimb glucose uptake but not between femoral blood flow and glucose uptake. The results indicate that in vivo, certain types of vasoconstriction in muscle such as elicited by 5HT$_2$ agonists, which prevent normal insulin recruitment of capillary flow, cause impaired muscle glucose uptake. Moreover, if vasoconstriction of this kind results from stress-induced increase in sympathetic outflow, then this may provide a clue as to the link between hypertension and insulin resistance that is often observed in humans. ***Diabetes*** **48:564–569, 1999**

Apart from its metabolic actions on skeletal muscle, insulin is now recognized to have major actions on the skeletal muscle's vascular system (1). Insulin increases total blood flow to skeletal muscle by causing an NO-dependent vasodilatation within the vasculature (2–4). Insulin also causes changes in muscle blood volume (5) and capillary recruitment (6). In many insulin-resistant states such as hypertension, obesity, NIDDM, IDDM, and aging, the vascular actions of insulin to increase total blood flow in muscle are impaired (7–11). These results have led to the proposal that impaired insulin action on the vascular system can contribute to the insulin resistance in these states (12–14).

In the in vitro perfused rat hindlimb system, muscle metabolism is regulated by site-specific vasoconstriction (15,16). Muscle metabolism and aerobic contraction performance were either increased or decreased depending on the vasoconstrictor used (15). These changes in metabolism during constant flow were associated with alterations in capillary flow routes within muscle and not arteriovenous shunts or changes in flow distribution among different muscle types or among muscle and nonmuscle tissue within the hindlimb (17,18). Vasoconstriction that led to decreases in metabolism and nutritive blood flow also reduced insulin-mediated glucose uptake, resulting in acute insulin resistance (19,20). Vasoactive agents that acted in this manner included serotonin agonists, norepinephrine at doses that reflect those obtained at sympathetic vasocontrictor synapses, and high- frequency sympathetic nerve stimulation (15). In addition, the vasoconstrictor-induced effects on metabolism, whether stimulatory or inhibitory, were opposed by vasodilators (13). Thus vasodilators are also capable of having inhibitory and stimulatory effects on muscle metabolism. These results indicate the importance of the vascular system in regulating nutrient and hormone access to muscle and further support the notion that impaired vascular action can contribute to insulin resistance (12,13).

We have recently developed a method to measure muscle capillary flow both in vitro and in vivo (6,18). The method is based on the metabolism of 1-methylxanthine (1-MX) by xanthine oxidase located in the capillaries (21,22). Under conditions of constant flow to the hindlimb, serotonin vasoconstriction that had an inhibitory effect on metabolism and caused acute insulin resistance also markedly decreased metabolism of 1-MX (18). Serotonin had no direct effect, however, on xanthine oxidase activity and caused no

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A, arterial; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; FBF, femoral blood flow; HPLC, high-performance liquid chromatography; α-met5HT, α-methylserotonin; 1-MX, 1-methylxanthine; RU, resistance unit; V, venous.

changes in total flow to each muscle (18). We observed in vivo that during a euglycemic insulin clamp in anesthetized rats, 1-MX metabolism was increased in association with increases in total muscle blood flow and glucose metabolism. Because insulin did not directly activate xanthine oxidase and increased 1-MX uptake was not simply due to increased total flow, it is likely that insulin caused capillary recruitment, along with vasodilatation, in muscle (6).

In the present study, the relationship between blood flow and insulin-mediated glucose metabolism in muscle was further investigated in vivo using the vasoactive agent α-methylserotonin (α-met5HT). When administered intravenously, α-met5HT is a serotonergic agonist that stimulates $5HT_2$ receptors (23) in the periphery but does not act on central nervous system (CNS) serotonin receptors (24). α-Met5HT, since it acts via the same receptors on the vasculature as serotonin, decreases muscle metabolism in the constant-flow perfused rat hindlimb (25) and thus would be expected to decrease 1-MX metabolism, an indicator of muscle capillary flow. Therefore, α-met5HT administration in vivo was used to assess the importance of capillary recruitment for insulin-mediated glucose metabolism in skeletal muscle and to test the hypothesis that vasoconstriction known to diminish capillary flow in muscle in vitro would generate hypertension and impair insulin-mediated glucose metabolism in vivo.

RESEARCH DESIGN AND METHODS

Animals. Male Sprague-Dawley rats weighing 294 ± 7 g (n = 32) were obtained from Hilltop Laboratory Animals (Scotdale, PA) and studied ≥3 days after arrival. Animals were housed under controlled temperature (22 ± 2°C) and lighting (12 h of light, 0600–1800; 12 h of dark, 1800–0600) with free access to water and standard rat food. The animal research committee of the University of Virginia approved all procedures.

Surgery. Rats were anesthetized (Nembutal, 50 mg/kg body wt) and had polyethylene (PE 50) (Intramedic) cannulas surgically implanted into the carotid artery for arterial sampling and measurement of blood pressure (pressure transducer Transpac IV; Abbott Critical Systems, North Chicago, IL), and the jugular vein for intravenous infusions. A tracheostomy tube was placed, and the animal was allowed to breathe room air spontaneously during the course of the experiment. Small incisions (1.5 cm) were made in the skin overlaying the femoral vessels of both legs, and the femoral artery was separated from femoral vein and saphenous nerve. After the epigastric vessels were ligated, a flow probe (VB series 0.5 mm; Transonic Systems, Ithaca, NY) was positioned around the femoral artery just distal to the rectus abdominis muscle of the left leg and connected to the flow meter (model T106 ultrasonic volume flow meter; Transonic Systems, Ithaca, NY). The flow meter was in turn interfaced with an IBM-PC compatible computer that acquired the data (100 Hz sampling frequency) for femoral blood flow, heart rate, and blood pressure using WINDAQ data acquisition software (DATAQ Instruments, Akron, OH). The femoral vein of the right leg was used for venous sampling. Typically, the surgical procedure required 30 min and then the animals were maintained under anesthesia throughout the experiment by continual infusion of Nembutal (0.6 mg · min^{-1} · kg^{-1}) via the arterial cannula. A heat lamp positioned above the rat maintained body temperature.

Experimental protocols. Completion of the surgical procedures was followed by a 60-min baseline period to allow leg blood flow and blood pressure to become constant. Rats were then divided into either control (saline plus met5HT) or euglycemic insulin clamp (insulin alone or α-met5HT plus insulin) groups (n = 8 in each group) and underwent the two protocols outlined in Fig. 1. Saline and met5HT infusions in the control group were matched to the volumes of insulin (Humulin R; Eli Lilly, Indianapolis) and glucose infused in the euglycemic insulin clamp groups. As reported previously (6), we observed that 1-MX clearance was very rapid, and it was necessary to partially inhibit the xanthine oxidase activity with allopurinol (10 μmol/kg), a specific xanthine oxidase inhibitor (26). Total blood volume drawn before the femoral arterial (A) and venous (V) samples at the end of the experiment did not exceed 1.5 ml and was easily compensated by the volume of fluid infused.

Duplicate A and V samples (250 μl) taken at the end of the experiment (120 min) were immediately centrifuged, and 100 μl plasma was mixed with 20 μl of 2 mol/l perchloric acid. The perchloric acid–treated samples were then centrifuged, and the supernatant was stored at –20°C until assayed for 1-MX. The rest of the plasma was collected and used for insulin analysis.

Analysis procedures. Whole blood was measured by the glucose oxidase method on a glucose analyzer (Model 23A; Yellow Springs Instruments, Yellow Springs, OH). Basal rat insulin and human insulin levels during the euglycemic insulin clamp were determined from arterial plasma samples by radioimmunoassay, using rat or human insulin standards where appropriate. 1-MX, allopurinol, and oxypurinol were determined in the perchloric acid extracts of plasma by reverse-phase high-performance liquid chromatography (HPLC) essentially as described by Wynants et al. (27). 1-MX, allopurinol, and oxypurinol were separated on an Ultrasphere ODS column (25-cm; 5-μm particles; Beckman, Fullerton, CA) under isocratic conditions at 1.0 ml/min with 0.3 mol/l KH_2PO_4, 0.5% methanol, 0.5% acetonitrile, 0.2% tetrahydrofuran, pH 4.0, buffer. Detection was performed at wavelengths of 254 and 280 nm.

Data analysis. All data are presented as means ± SE. Mean femoral blood flow, mean heart rate, and mean arterial pressure were calculated from 5-s subsamples of the data, representing 500 flow and pressure measurements of 15 min each. Vascular resistance in the hindlimb was calculated as mean arterial blood pressure in millimeters of mercury divided by femoral blood flow in milliliters per minute and expressed as resistance units (RU). Glucose uptake in the hindlimb was calculated from A – V glucose difference and multiplied by femoral blood flow and expressed as micromoles per minute. 1-MX disappearance was calculated from A – V plasma 1-MX difference multiplied by femoral blood flow (corrected for the volume accessible to 1-MX, 0.871, determined from plasma concentrations obtained after additions of standard 1-MX to whole rat blood) and expressed as nanomoles per minute.

Statistical analysis. Repeated measures analysis of variance was used for differences between treatment groups. When a significant difference ($P < 0.05$) was found, Dunnett's test was used to determine which times were significantly different from t = 0. Statistical difference between treatments for femoral blood flow, arterial blood pressure, femoral vascular resistance, arterial glucose and 1-MX, hindlimb glucose extraction and uptake, and hindlimb 1-MX extraction and dis-

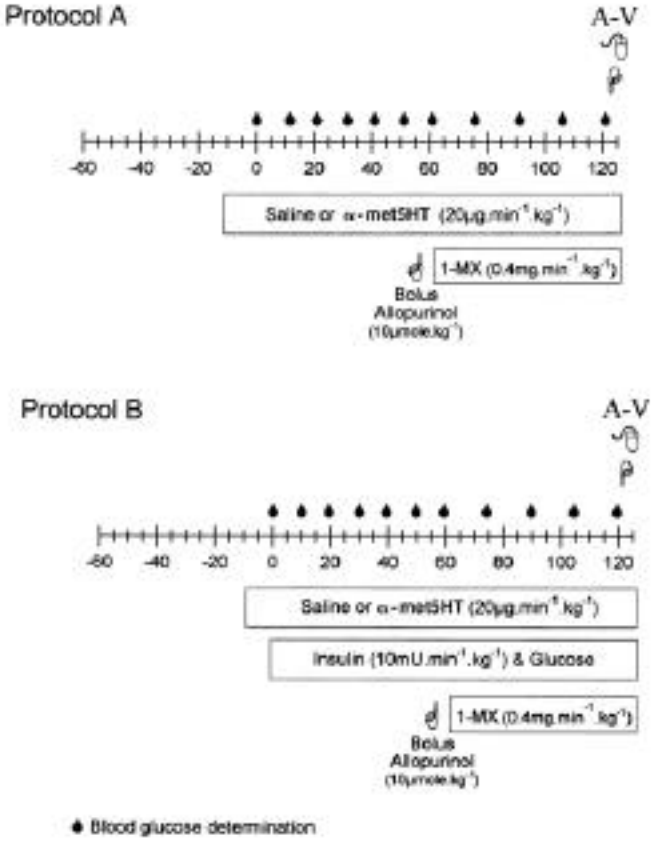

FIG. 1. Study design. In the control groups (protocol A), time 0 was the start of saline or α-met5HT infusions. In the euglycemic insulin clamp groups (protocol B), time 0 was the start of the insulin infusion. Duplicate arterial and femoral venous blood samples were collected at 120 min as indicated by 8 h for HPLC analysis and arterial samples for glucose determination by ♦. Venous infusions are indicated by the bars.

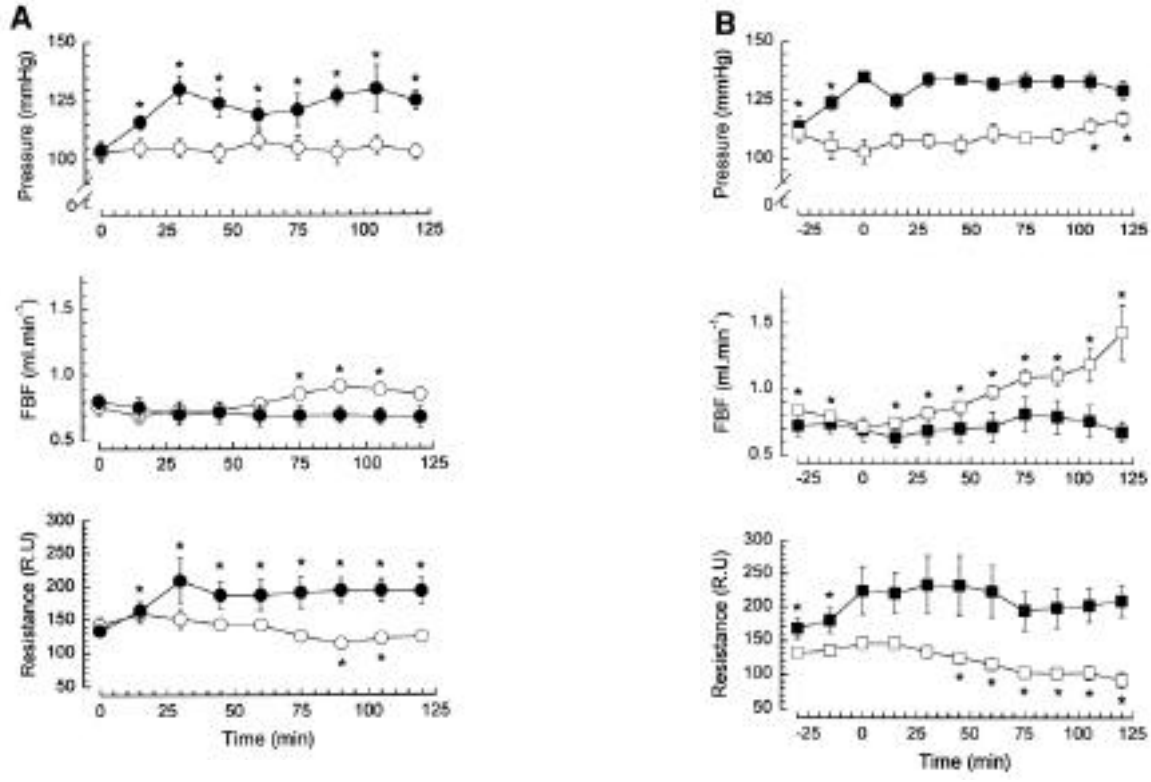

FIG. 2. Systemic and hindlimb changes in hemodynamic factors. *A*: Rats treated with saline (○) and α-met5HT (●) as described in protocol A. *B*: Rats treated with insulin (□) and α-met5HT plus insulin (■) as described in protocol B. Data were collected from 5-s subsamples each 15 min as described in METHODS. Values are means ± SE for eight animals in each group. *Significantly different ($P < 0.05$) from 0 time.

appearance at the end of the experiment was determined by two-way analysis of variance and appropriate post hoc testing. These tests were performed using the SigmaStat statistical program (Jandel Scientific, San Rafael, CA).

RESULTS

Hemodynamic effects. Figure 2 shows the arterial blood pressure, femoral blood flow (FBF), and hindlimb vascular resistance after saline or α-met5HT infusions during protocol A (Fig. 2*A*) and after insulin or α-met5HT plus insulin infusions during the euglycemic insulin clamps in protocol B (Fig. 2*B*). α-Met5HT infusion significantly increased ($P < 0.05$) arterial blood pressure within 30 min by 25%, a level maintained throughout the infusion whether insulin was also infused or not (Fig. 2). The α-met5HT-mediated increase in blood pressure was associated with significant increases in hindlimb vascular resistance but no significant change in femoral blood flow (Fig. 2*A*). Insulin alone caused a significant increase ($P < 0.05$) in femoral blood flow associated with decreases in hindlimb vascular resistance (Fig. 2*B*). By the end of the experiment, femoral blood flow had increased by 70% and hindlimb vascular resistance had decreased by 30%. The insulin-mediated increase in femoral blood flow and decrease in hindlimb vascular resistance was completely prevented during infusion with α-met5HT (Fig. 2*B*). α-Met5HT infusion, alone or with insulin infusion, caused a significant ($P < 0.05$) increase in heart rate (α-met5HT alone, 371 ± 12 to 445 ± 17 beats/min; α-met5HT plus insulin, 348 ± 23 to 417 ± 14 beats/min) by the end of the experiment. Saline and insulin infusions alone had no significant effect on heart rate (saline, 327 ± 10 to 295 ± 13 beats/min; insulin, 388 ± 18 to 332 ± 13 beats/min).

Glucose metabolism. There was a small (1.36 ± 0.29 mmol/l) significant increase in blood glucose by the end of the saline infusion. No significant differences were observed for blood glucose values during α-met5HT infusion (data not shown). Blood glucose was maintained at or above basal values during the euglycemic insulin clamp experiments by glucose infusion. The glucose infusion rate was significantly higher ($P < 0.05$) at all times during the infusions of insulin alone compared with the infusions of α-met5HT plus insulin. Steady-state rates were 24.63 ± 0.90 and 17.67 ± 1.21 mg · min^{-1} · kg^{-1} for insulin alone and α-met5HT plus insulin, respectively.

Basal plasma insulin concentrations (396 ± 104 pmol/l) were not significantly different between groups. Saline and α-met5HT infusions did not result in significantly different plasma insulin values at the end of the experiment. Insulin plasma concentrations were significantly increased ($P < 0.001$) at the end of the euglycemic insulin clamp, and the plasma values were significantly higher ($P < 0.05$) in the α-met5HT plus insulin experiments (2,625 ± 197 pmol/l) than the insulin alone experiments (1,740 ± 211 pmol/l).

Hindlimb glucose extraction and uptake were significantly increased ($P < 0.001$) by the euglycemic insulin clamps (Fig. 3). α-Met5HT infusion had no effect alone on hindlimb glucose extraction and uptake, but it significantly reduced ($P < 0.05$) the insulin-mediated hindlimb glucose uptake without affecting hindlimb glucose extraction (Fig. 3).

1-MX metabolism. No significant difference was found between the experimental groups in arterial plasma concentrations of 1-MX (Fig. 4) or oxypurinol (saline, 5.65 ± 0.70;

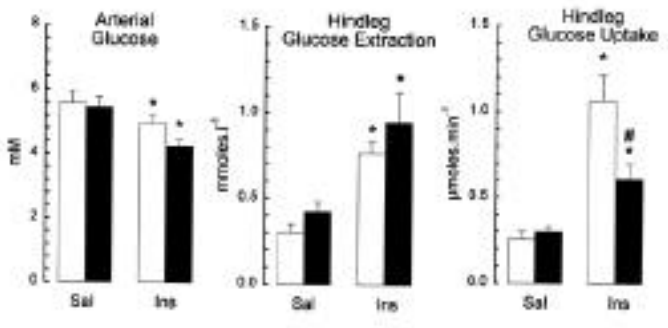

FIG. 3. Systemic and hindlimb glucose values of control groups (Sal), saline and α-met5HT, and euglycemic insulin clamp groups (Ins), insulin and α-met5HT plus insulin. Groups infused with α-met5HT are shown as filled bars. Values are means ± SE for eight animals in each group. *Significantly different ($P < 0.05$) from saline values. #Significantly different ($P < 0.05$) from corresponding untreated group.

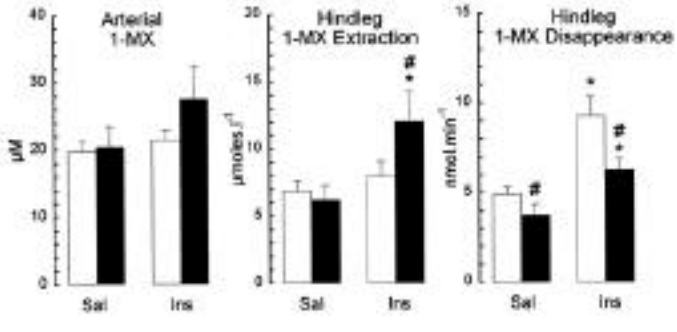

FIG. 4. Systemic and hindlimb 1-MX values of control groups (Sal), saline and α-met5HT, and euglycemic insulin clamp groups (Ins), insulin and α-met5HT plus insulin. Groups infused with α-met5HT are shown as filled bars. Values are means ± SE for eight animals in each group. *Significantly different ($P < 0.05$) from saline values. #Significantly different ($P < 0.05$) from corresponding untreated group.

α-met5HT, 5.43 ± 0.71; insulin, 4.58 ± 0.25; and α-met5HT plus insulin, 4.93 ± 0.35 μmol/l), the metabolite of allopurinol and inhibitor of xanthine oxidase.

Hindlimb metabolism of 1-MX was significantly ($P < 0.05$) increased during the euglycemic insulin clamps. The changes to hindlimb 1-MX extraction with α-met5HT infusion alone were not significant; when combined with the changes in flow, however, the α-met5HT infusion caused significant ($P < 0.05$) decreases in hindlimb 1-MX metabolism (Fig. 4). There was a 24% decrease in hindlimb 1-MX metabolism by α-met5HT alone and a 71% reduction in the insulin-mediated increase in hindlimb 1-MX metabolism by α-met5HT.

DISCUSSION

Systemic administration of α-met5HT to anesthetized rats led to increased peripheral vascular resistance and decreased whole body insulin-stimulated glucose uptake and hindlimb glucose uptake. The inhibition of insulin-stimulated glucose metabolism was associated with inhibition of insulin-mediated increases in total femoral blood flow and hindlimb 1-MX metabolism, indicative of decreased capillary blood flow. These findings highlight the importance of vascular actions for the normal stimulation of muscle glucose metabolism by insulin.

We chose α-met5HT as the vasoconstrictor in these experiments, since it was one of the vasoactive agents that caused decreased muscle metabolism in the constant-flow surgically isolated perfused rat hindlimb (25). It had the advantage in vivo that no direct receptor-mediated metabolic action would occur on skeletal muscle. α-Met5HT is a serotonergic agonist that stimulates $5HT_2$ receptors and has high affinity for both vasoconstricting $5HT_{2A}$ and vasodilating $5HT_{2B}$ receptors in the vasculature (23). Unlike other $5HT_2$ agonists, such as DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] or naturally occurring serotonin, α-met5HT does not cross the blood-brain barrier and thus in vivo does not have the complicating actions of stimulating the numerous classes of 5HT receptors in the brain (24). Large bolus infusions (0.5–1.0 mg/kg i.v.) of α-met5HT in vivo have been reported to cause hyperglycemia and increases in plasma insulin concentrations in rats (28). The constant infusion (20 $\mu g \cdot min^{-1} \cdot kg^{-1}$) of α-met5HT used in this study did not cause hyperglycemia or increase plasma insulin concentrations. This dose of α-met5HT did lead to significant increases in mean arterial blood pressure and femoral vascular resistance (Fig. 2), but femoral blood flow was not significantly affected. This finding is consistent with the finding by Chaouche-Teyara et al. (29), who demonstrated that both $5HT_2$ agonists DOI and α-met5HT increased blood pressure and total peripheral resistance in anesthetized rats. In contrast, serotonin administration in vivo caused decreased blood pressure and vascular resistance, resulting in increased leg blood flow (30). Thus it appears that α-met5HT stimulation of the vasoconstrictive $5HT_{2A}$ receptors predominates over that of the $5HT_{2B}$ receptors or that serotonin has central effects at other receptor sites that oppose the peripheral $5HT_{2A}$ stimulation.

Physiologically, it is unlikely that peripheral serotoninergic mechanisms are involved in insulin resistance associated with essential hypertension (31), although such mechanisms may be involved in infection or shock where serotonin release occurs from platelets (32). Judging from perfused rat hindlimb studies, however, the sites of action in the vasculature are likely to be the same as those by other vasoactive agents that are involved in hypertension (15).

As reported previously by us (6), insulin administration during euglycemic insulin clamp resulted in significant increases in femoral blood flow in association with decreased femoral vascular resistance and slightly increased blood pressure (Fig. 2*B*). Because of the need to monitor total hindlimb flow and to sample femoral vein blood without affecting normal blood flow, the experiments were conducted in anesthetized rats. Thus anesthesia-induced decrease in muscle blood flow (33) may have led to confounding effects. However, these changes in anesthetized rats are similar in magnitude and in time of onset to those observed for changes caused by insulin in human skeletal muscle (1,34–38). These vascular effects of insulin were completely inhibited by α-met5HT administration begun before the insulin clamp. In addition, insulin was unable to reverse the increase in vascular resistance induced by α-met5HT, and femoral blood flow remained unchanged (Fig. 2*B*). The action of insulin in these animals reflects the situation observed on insulin administration to some (9,39–41) but not all (42) hypertensive humans, where no decrease in vascular resistance is observed.

Insulin vasodilatation is NO-dependent (2–4), and $5\text{-}HT_{2B}$–mediated vasodilatation by serotonin has also been demonstrated to be NO-dependent (43). α-Met5HT infusion

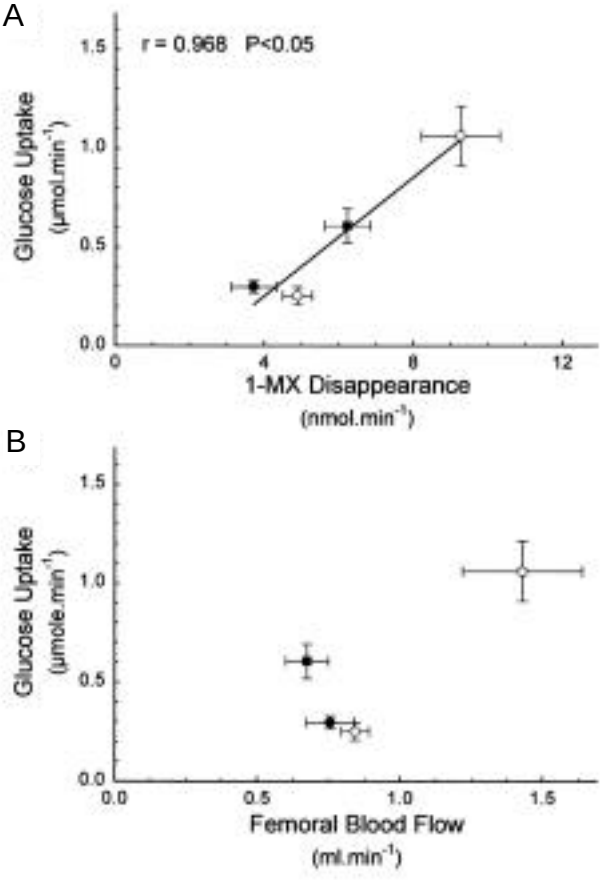

FIG. 5. Relationships between hindlimb 1-MX metabolism and glucose uptake (*A*) and femoral blood flow and glucose uptake (B). Rats were treated with saline (○) and α-met5HT (●), as described in protocol A, and with insulin (□) and α-met5HT plus insulin (■), as described in protocol B. Values are means ± SE for eight animals in each group.

in the present experiments caused increased vascular resistance, indicating that the constrictive actions of α-met5HT overwhelmed any NO-mediated vasodilatation and thus may account for why insulin was ineffective in increasing femoral blood flow in the presence of α-met5HT. The vascular sites of constriction by α-met5HT may also be important. Serotonin constricts large arterioles (44,45) but not small arterioles (30,46), suggesting that constriction occurs at the feed arteries external to the muscle fibers. In the constant-flow perfused rat hindlimb, serotonin-mediated vasoconstriction that causes acute insulin resistance is also resistant to contraction-induced metabolic vasodilatation (47) and may preferentially affect flow within muscles rich in type II fibers (20). Insulin infusion in the present study and previously published studies (6) caused increases in 1-MX metabolism (Fig. 4), indicating greater capillary recruitment and suggesting that insulin's vascular actions occur within the muscle and not at the feed arteries. Thus insulin's inability to overcome the vasoconstriction by α-met5HT (Fig. 2*B*) may be a result of differences in the vascular sites of action by these two agents, which may also differ within the various muscles.

Previous studies in the perfused rat hind limb and isolated incubated muscles have demonstrated that serotonin-mediated inhibition of insulin-stimulated glucose uptake is not due to any direct action of serotonin on muscle but is due to its vascular actions (20). Changes in insulin-mediated glucose uptake during constant flow in the perfused hindlimb were associated with alterations in capillary flow routes within muscle and not arteriovenous shunts or changes in flow distribution among different muscle types or among muscle and nonmuscle tissue (17,18). Similar mechanisms appear to occur in vivo, since both the insulin increases in total blood flow to the hindlimb (Fig. 2*B*) and the capillary flow (Fig. 4) were inhibited by α-met5HT, resulting in decreased insulin-mediated hindlimb glucose uptake (Fig. 3). There was no increase in blood glucose concentrations when α-met5HT was infused alone, indicating that the reduced glucose infusion rate to maintain euglycemia during insulin and α-met5HT infusion was not due to stimulation of hepatic glucose output but to the decreased whole body muscle glucose uptake.

Interestingly, α-met5HT infusion alone decreased capillary recruitment without affecting total flow or hindlimb glucose uptake (Fig. 3). Similarly, in the constant-flow perfused rat hind limb, serotonin did not affect basal glucose uptake (20) even though 1-MX metabolism, indicating capillary flow, and oxygen uptake were reduced (18). Thus it appears that nutrient delivery is sufficient for basal muscle glucose uptake even with diminished capillary flow but that nutrient and/or insulin delivery is crucial when muscle glucose metabolism is stimulated by insulin. A significant correlation ($P < 0.05$) was found between hindlimb glucose uptake and 1-MX metabolism but not between hindlimb glucose uptake and femoral blood flow (Fig. 5). Thus measurement of only total blood flow is not sufficient to determine whether there is a hemodynamic contribution of insulin to its metabolic actions, and consideration of changes in blood flow distribution at a capillary level are more important.

Studies in the perfused hindlimb have demonstrated that many other vasoactive agents (such as norepinephrine) and high-frequency sympathetic nervous system stimulation have the same effect on metabolism as serotonin, and they appear to act at the same vascular sites as serotonin (15). α-Met5HT was chosen in this study because it is a vasoconstrictor agent that does not have direct effects on muscle metabolism, but its actions are restricted to the vasculature. The results indicate that in anesthetized rats in vivo, vasoconstriction in muscle that prevents normal insulin recruitment of capillary flow causes impaired muscle glucose uptake, despite there being no change in total flow. Such mechanisms may be important in establishing the link between hypertension and insulin resistance that is often observed in humans. Not all vasoconstriction will necessarily lead to these effects; for example, vasoconstriction mediated by angiotensin has been reported to improve insulin sensitivity in vivo (48) and increases metabolism in the constant-flow perfused rat hindlimb (15).

In summary, the findings represent the first report of acute insulin resistance associated with reduced capillary recruitment in muscle resulting from vasoconstriction actions in vivo where systemic rise in blood pressure occurred without a change in total blood flow.

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REFERENCES

1. Baron AD: Hemodynamic actions of insulin. *Am J Physiol* 267:E187–E202, 1994
2. Baron AD: Nitric oxide mediates skeletal glucose transport (Reply). *Am J Physiol* 270:E1058–E1059, 1996
3. Chen YL, Messina EJ: Dilation of isolated skeletal muscle arterioles by insulin is endothelium dependent and nitric oxide mediated. *Am J Physiol* 270:H2120–H2124, 1996
4. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511–2515, 1994
5. Raitakari M, Knuuti MJ, Ruotsalainen U, Laine H, Mäkelä P, Teräs M, Sipilä H, Niskanen T, Raitakari OT, Iida H, Härkönen R, Wegelius U, Yki-Järvinen H, Nuutila P: Insulin increases blood volume in human skeletal muscle: Studies using [^{15}O]CO and positron emission tomography. *Am J Physiol* 269:E1000–E1005, 1995
6. Rattigan S, Clark MG, Barrett EJ: Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* 46:1381–1388, 1997
7. Baron AD, Laakso M, Brechtel G, Hoit B, Watt C, Edelman SV: Reduced postprandial skeletal muscle blood flow contributes to glucose intolerance in human obesity. *J Clin Endocrinol Metab* 70:1525–1533, 1990
8. Baron AD, Laakso M, Brechtel G, Edelman SV: Mechanism of insulin resistance in insulin-dependent diabetes mellitus: a major role for reduced skeletal muscle blood flow. *J Clin Endocrinol Metab* 73:637–643, 1991
9. Baron AD, Brechtel-Hook G, Johnson A, Hardin D: Skeletal muscle blood flow. A possible link between insulin resistance and blood pressure. *Hypertension* 21:129–135, 1993
10. Laakso M, Edelman SV, Brechtel G, Baron AD: Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–1083, 1992
11. Meneilly GS, Elliot T, Bryer-Ash M, Floras JS: Insulin-mediated increase in blood flow is impaired in the elderly. *J Clin Endocrinol Metab* 80:1899–1903, 1995
12. Baron AD: Insulin and the vasculature: old actors, new roles. *J Invest Med* 44:406–412, 1996
13. Baron AD, Clark MG: Role of blood flow in the regulation of muscle glucose uptake. *Annu Rev Nutr* 17:487–499, 1997
14. Baron AD: The coupling of glucose metabolism and perfusion in human skeletal muscle: the potential role of endothelium-derived nitric oxide. *Diabetes* 45 (Suppl. 1):S105–S109, 1996
15. Clark MG, Colquhoun EQ, Rattigan S, Dora KA, Eldershaw TP, Hall JL, Ye J: Vascular and endocrine control of muscle metabolism (Review). *Am J Physiol* 268:E797–E812, 1995
16. Dora KA, Richards SM, Rattigan S, Colquhoun EQ, Clark MG: Serotonin and norepinephrine vasoconstriction in rat hindlimb have different oxygen requirements. *Am J Physiol* 262:H698–H703, 1992
17. Newman JMB, Dora KA, Rattigan S, Edwards SJ, Colquhoun EQ, Clark MG: Norepinephrine and serotonin vasoconstriction in rat hindlimb control different vascular flow routes. *Am J Physiol* 270:E689–E699, 1996
18. Rattigan S, Appleby GJ, Miller KA, Steen JT, Dora KA, Colquhoun EQ, Clark MG: Serotonin inhibition of 1-methylxanthine metabolism parallels inhibition of oxygen uptake in perfused rat hindlimb. *Acta Physiol Scand* 161:161–169, 1997
19. Rattigan S, Dora KA, Colquhoun EQ, Clark MG: Inhibition of insulin-mediated glucose uptake in rat hindlimb by an α-adrenergic vascular effect. *Am J Physiol* 268:E305–E311, 1995
20. Rattigan S, Dora KA, Colquhoun EQ, Clark MG: Serotonin-mediated acute insulin resistance in the perfused rat hindlimb but not in incubated muscle: a role for the vascular system. *Life Sci* 53:1545–1555, 1993
21. Parks DA, Granger DN: Xanthine oxidase: biochemistry, distribution and physiology (Review). *Acta Physiol Scand* 548 (Suppl.):87–99, 1986
22. Jarasch ED, Bruder G, Heid HW: Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol Scand* 548 (Suppl.):39–46, 1986
23. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP: International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 46:157–203, 1994
24. Baudrie V, Chaouloff F: Mechanisms involved in the hyperglycemic effect of the 5-HT1C/5-HT2 receptor agonist, DOI. *Eur J Pharmacol* 213:41–46, 1992
25. Newman JMB, Clark MG: Stimulation and inhibition of resting muscle thermogenesis by vasoconstrictors in perfused rat hindlimb. *Can J Physiol Pharmacol.* (In press)
26. Emmerson BT, Gordon RB, Cross M, Thomson DB: Plasma oxipurinol concentrations during allopurinol therapy. *Br J Rheumatol* 26:445–449, 1987
27. Wynants J, Petrov B, Nijhof J, Van Belle H: Optimization of a high-performance liquid chromatographic method for the determination of nucleosides and their catabolites: application to cat and rabbit heart perfusates. *J Chromatogr* 386:297–308, 1987
28. Chaouloff F, Laude D, Baudrie V: Effects of the 5-HT1C/5-5-HT2 receptor agonists DOI and alpha-methyl-5-HT on plasma glucose and insulin levels in the rat. *Eur J Pharmacol* 187:435–443, 1990
29. Chaouche-Teyara K, Fournier B, Safar M, Dabire H: Systemic and regional haemodynamic effects of 1-(2,5-dimethoxy-4-iodo-phenyl)-2-aminopropane (DOI) and alpha-methyl-5-HT, in the anaesthetised rat. *Clin Exp Hypertens* 16:779–798, 1994
30. Blackshear JL, Orlandi C, Garnic JD, Hollenberg NK: Differential large and small vessel responses to serotonin in the dog hindlimb in vivo: role of the 5HT2 receptor. *J Cardiovasc Pharmacol* 7:42–49, 1985
31. Van Zwieten PA, Blauw GJ, van Brummelen P: The role of 5-hydroxytryptamine and 5-hydroxytryptaminergic mechanisms in hypertension. *Br J Clin Pharmacol* 30 (Suppl 1):69S–74S, 1990
32. Schiffrin EL: The endothelium and control of blood vessel function in health and disease. *Clin Invest Med* 17:602–620, 1994
33. James DE, Burleigh KM, Storlien LH, Bennett SP, Kraegen EW: Heterogeneity of insulin action in muscle: influence of blood flow. *Am J Physiol* 251:E422–E430, 1986
34. Baron AD, Brechtel G: Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am J Physiol* 265:E61–E67, 1993
35. Vierhapper H, Gasic S, Roden M, Waldhausl W: Increase in skeletal muscle blood flow but not in renal blood flow during euglycemic hyperinsulinemia in man. *Horm Metab Res* 25:438–441, 1993
36. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL: Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 87:2246–2252, 1991
37. Utriainen T, Malmström R, Mäkimattila S, Yki-Järvinen H: Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. *Diabetologia* 38:555–564, 1995
38. Vollenweider L, Tappy L, Owlya R, Jéquier E, Nicod P, Scherrer U: Insulin-induced sympathetic activation and vasodilation in skeletal muscle: effects of insulin resistance in lean subjects. *Diabetes* 44:641–645, 1995
39. Feldman RD, Bierbrier GS: Insulin-mediated vasodilation: impairment with increased blood pressure and body mass. *Lancet* 342:707–709, 1993
40. Fujishima S, Imaizumi T, Abe I, Takeshita A, Fujishima M: Effects of intra-arterial infusion of insulin on forearm vasoreactivity in hypertensive humans. *Hypertens Res* 18:227–233, 1995
41. Gudbjörnsdottir S, Elam M, Sellgren J, Anderson EA: Insulin increases forearm vascular resistance in obese, insulin-resistant hypertensives. *J Hypertens* 14:91–97, 1996
42. Hunter SJ, Harper R, Ennis CN, Sheridan B, Atkinson AB, Bell PM: Skeletal muscle blood flow is not a determinant of insulin resistance in essential hypertension. *J Hypertens* 15:73–77, 1997
43. Bruning TA, Chang PC, Blauw GJ, Vermeij P, Van Zwieten PA: Serotonin-induced vasodilatation in the human forearm is mediated by the "nitric oxide-pathway": no evidence for involvement of the 5-HT3-receptor. *J Cardiovasc Pharmacol* 22:44–51, 1993
44. Lamping KG, Kanatsuka H, Eastham CL, Chilian WM, Marcus ML: Nonuniform vasomotor responses of the coronary microcirculation to serotonin and vasopressin. *Circ Res* 65:343–351, 1989
45. Wilmoth FR, Harris PD, Miller FN: Differential serotonin responses in the skeletal muscle microcirculation. *Life Sci* 34:1135–1141, 1984
46. Hollenberg NK: Large and small vessel responses to serotonin in the peripheral circulation. *J Cardiovasc Pharmacol* 7 (Suppl. 7):S89–S91, 1985
47. Dora KA, Rattigan S, Colquhoun EQ, Clark MG: Aerobic muscle contraction impaired by serotonin-mediated vasoconstriction. *J Appl Physiol* 77:277–284, 1994
48. Buchanan TA, Thawani H, Kades W, Modrall JG, Weaver FA, Laurel C, Poppiti R, Xiang A, Hseuh W: Angiotensin II increases glucose utilization during acute hyperinsulinemia via a hemodynamic mechanism. *J Clin Invest* 92:720–726, 1993