HISS, not insulin, causes vasodilation in response to administered insulin

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Ming Z, Lautt WW. HISS, not insulin, causes vasodilation in response to administered insulin. J Appl Physiol 110: 60–68, 2011. First published September 9, 2010; doi:10.1152/japplphysiol.00714.2010.—Meal-induced sensitization to the dynamic actions of insulin results from the peripheral actions of a hormone released by the liver (hepatic insulin sensitizing substance or HISS). Absence of meal-induced insulin sensitization results in the pathologies associated with cardiometabolic risk. Using three protocols that have previously demonstrated HISS metabolic action, we tested the hypothesis that HISS accounts for the vasodilation that has been associated with insulin. The dynamic metabolic actions of insulin and HISS were determined using a euglycemic clamp in response to a bolus of 100 μU/kg insulin in pentobarbital-anesthetized Sprague-Dawley rats. Hindlimb blood flow was measured with an ultrasound flow probe on the aorta above the bifurcation of the iliac arteries. Fed rats showed tightly coupled metabolic and vascular responses, which were completed by 35 min after insulin administration. Blocking HISS release, with the use of atropine or hepatic surgical denervation, eliminated the HISS-dependent metabolic and vascular responses to insulin administration. Physiological suppression of HISS release occurs with fasting. In 24-h fasted rats, HISS metabolic and vascular actions were absent, and atropine had no effect on either action. Fed rats with liver denervation did not release HISS, but intraportal venous infusion of acetylcholine, to mimic the permissive parasympathetic nerve signal, restored the ability of insulin to cause HISS release and restored both the metabolic and vascular actions. These studies report vascular actions of HISS for the first time and demonstrate that HISS, not insulin action, results in the peripheral vasodilation generally attributed to insulin.

rapid insulin sensitivity test; blood flow; cardiometabolic risk; diabetes; hepatic insulin sensitizing substance

MICROVASCULAR DYSFUNCTION in numerous vascular beds is associated with prediabetes, diabetes, and obesity [reviewed previously (2, 22, 32)]. Impaired glucose tolerance is independently associated with macrovascular and microvascular dysfunction. The microvascular deficiency leads to polynuropathies, retinopathies, and nephropathy and is the leading cause of limb amputations.

Neuropathy is already present in 10–18% of patients at the time of diabetes diagnosis (8), and significant numbers of patients have nephropathy or retinopathy by the time diabetes is diagnosed (10, 24). Vascular complications are frequent in insulin-resistant patients even before they develop fasting hyperglycemia (11). In normoglycemic, normotensive individuals with a family history of diabetes, systemic insulin resistance correlates with blunted vasoreactivity, independent of hyperglycemia (3, 5).

Investigations into the progression of impaired glucose tolerance and endothelial dysfunction reveal a dynamic and reversible state. Vascular function appears to improve if glucose metabolism homeostasis is reestablished by diet or exercise, but the effects of oral hypoglycemics such as rosiglitazone (33) or anticholesterol drugs such as pravastatin seem to be without beneficial effect on flow-induced vasodilation in the forearm (1).

Local hyperinsulinemia in healthy lean individuals produced no change in forearm blood flow, whereas systemic hyperinsulinemia caused a 52% increase in blood flow, implicating an indirect action of insulin (6). Local forearm hyperinsulinemia did not result in vasodilation, whereas some indirect effect of systemically administered insulin resulted in vasodilation (6, 34). The vasodilator response to prolonged insulin infusion was present in fed but not fasted subjects (35, 37, 38).

We suggest that these studies are consistent with, and can be explained by, the hepatic insulin sensitizing substance (HISS) hypothesis [reviewed previously (12, 14, 18, 20)]. Although the chemical nature of HISS remains unknown, the metabolic action of a substance released from the liver and acting on skeletal muscle can be readily quantified. In vivo research tools and protocols have allowed determination of HISS action in health and disease. Pharmacological manipulation of HISS action has allowed exploration of the signaling pathways and conditions controlling HISS release. We now suggest that, in addition to quantifiable metabolic action, HISS may also have vascular action.

The dynamic metabolic response to insulin, assessed from the glucose disposal initiated by a bolus administration of insulin and measured using a euglycemic clamp, shows dramatic differences in the fed and fasted state. The glucose disposal effect of insulin is doubled in the fed state in rats and tripled in humans (29). Meal-induced insulin sensitization is the result of insulin causing the release from the liver of a putative hepatic hormone, referred to as HISS, which stimulates glucose uptake into skeletal muscle. HISS is only released, in response to insulin, in the fed state and only if two “feeding signals” are delivered to the liver (9) (Lautt, Schafer, Macedo, and Legare unpublished data, 2010). Both feeding signals play a permissive role in that they do not themselves result in altered glucose metabolism but allow a pulse of insulin to cause release of a pulse of HISS from the liver. One “feeding signal” is mediated by the hepatic parasympathetic nerves acting through ACh on muscarinic receptors (31), leading to activation of nitric oxide synthase (15). The second signal is an elevation in hepatic glutathione, which increases by 30–50% after a meal (9) (Lautt et al., unpublished data). When both feeding signals are present (9) (Lautt et al., unpublished data), the metabolic response to insulin is approximately doubled in rats and is increased almost threefold after a meal in humans (29). Meal-induced insulin sensitization is accounted for by HISS action. Absence of HISS action, and therefore of meal-induced insulin sensitization, results in nutrient storage.
shifting from glycogen in skeletal muscle to lipogenesis and adiposity (16, 17).

In this report, we test the hypothesis that HISS, and not insulin action, results in peripheral (hindlimb) vasodilatation. The metabolic and vascular actions of HISS and insulin are differentiated in several protocols, demonstrating no significant direct vasodilating effect of insulin but a dilation of blood vessels to the hindlimbs, which closely tracks the whole body metabolic action of HISS.

**MATERIALS AND METHODS**

All methods and care of the animals conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and the Guidelines of the Canadian Council on Animal Care. The Protocol Management and Review Committee at the University of Manitoba approved all protocols.

**Animals**

Male Sprague-Dawley rats (7 wk old, 200–225 g) were fed with a standard rat chow diet with free access to water for 2 wk to adapt to the housing environment. All rats, except those specifically mentioned, underwent an 8-h fast overnight followed by a refeeding period of 2 h immediately before surgical preparation. One group of rats underwent a 24-h fast process before surgery.

**General Surgical Preparation**

The rats were anesthetized with an intraperitoneal injection of pentobarbitonal sodium (54.7 mg/kg; CEVA Sante Animal, Libourne, France). The rats were placed on a temperature-controlled surgical table, and rectal temperature was monitored and held at 37–37.5°C. Spontaneous respiration was allowed through a tracheal catheter.

A vascular shunt between the left carotid artery and the left jugular vein was established, as previously described (19), for monitoring mean arterial blood pressure (MAP) and blood glucose level and for intravenous drug delivery. Flowing blood within the shunt ensures the real-time measurement of arterial blood glucose concentration, which is essential for the dynamic euglycemic clamp test as mentioned below. Animals were heparinized (100 IU/kg) to prevent clotting in the vascular shunt.

**Hindlimb Blood Flow Measurement**

Laparotomy was performed via a vertical midline abdominal incision. For monitoring arterial blood flow, the hindlimbs (HLBF), the abdominal aorta was isolated from the surrounding tissue at the level above bifurcation of the iliac arteries. An ultrasonic perivascular V-type flow probe (size 1.5 mm) was installed. The blood flow signals were collected with a small animal flow meter (T206, Transonic System, Ithaca, NY) and recorded and analyzed with IOX digital system (EMKA Technologies, Falls Church, VA). A fine-gauge hypodermic needle (30 × 1 in.) was inserted into the abdominal aorta at the level 1 cm above the flow probe to allow intra-arterial infusion of the vasodilators specifically to hindlimb vascular beds. The hindlimb vascular conductance (HLVC) is calculated as HLVC = HLBF/MAP.

**Acute Surgical Hepatic Vagotomy**

The visible nerves entering the liver in association with the hepatic common artery were isolated and transected. In addition, these areas were painted with 10% phenol in 95% ethanol solution. In the corresponding control groups, phenol was painted on the abdominal muscle to rule out the possible side effects of phenol.

**Rapid Insulin Sensitivity Test**

The rapid insulin sensitivity (RIST) was performed as previously described (19) except that an insulin dose of 100 mU/kg was used instead of the standard dose of 50 mU/kg. The baseline glucose levels were determined by samples taken from the arterial side of the vascular loop at 5-min intervals and continued until three successive stable determinations were made. The mean of these three data points was used as the baseline for the RIST. To perform the RIST, human biosynthetic insulin (100 mU/kg in 0.5 ml saline) was infused into the jugular vein at the rate of 0.1 ml/min for 5 min. After 1 min of insulin infusion, the first test glucose sample was determined, and a variable glucose infusion (10% in saline solution) was initiated. Blood samples were taken every 2 min, and the glucose infusion rate was adjusted to maintain euglycemia. The RIST index was the amount of glucose (mg/kg) infused, to maintain euglycemia, over the test period, which was terminated when no further glucose infusion was required (~35 min).

**Animal Groups and Protocols**

The hypothesis tested in these protocols is that HISS, not insulin, causes vasodilation in response to administered insulin.

**Protocol 1, vascular response to insulin in fed rats: effect of atropine.** This protocol was designed to test the prediction that vasodilation would occur during the control RIST (where HISS is released) but not after atropine where HISS release is blocked. Experiments were performed in rats (n = 12) that had undergone an 8-h fast followed by a 2-h refeeding before surgery to ensure that testing was done in the fed state. A control RIST was performed, and the changes in MAP, HLBF, and heart rate were monitored at 5-min intervals until the completion of the RIST. Then, the endothelium-dependent vasodilator, ACh, and the endothelium-independent vasodilator, sodium nitroprusside (SNP), were injected intra-arterially into the hindlimb vasculature via the cannula in the lower abdominal aorta (doses of 1 and 3 μg/kg for both chemicals, dissolved in 0.05 ml saline). Blood flow and arterial pressure were monitored. Atropine at the dose of 1 mg/kg was given intravenously (0.1 ml/min for 5 min) thereafter to block hepatic parasympathetic nervous activity. After atropine administration, all tests mentioned above were repeated.

**Table 1. Baseline values in animal groups with different treatments**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>N</th>
<th>Blood Glucose, mg%</th>
<th>MAP, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>HLBF, ml/min</th>
<th>HLVC, ml²/mmHg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed control</td>
<td>12</td>
<td>119 ± 4</td>
<td>103 ± 3</td>
<td>400 ± 6</td>
<td>13.4 ± 0.7</td>
<td>0.131 ± 0.007</td>
</tr>
<tr>
<td>Fed post-atropine</td>
<td>12</td>
<td>122 ± 5</td>
<td>99 ± 3</td>
<td>392 ± 6</td>
<td>12.4 ± 0.6</td>
<td>0.131 ± 0.010</td>
</tr>
<tr>
<td>Fasted control</td>
<td>8</td>
<td>102 ± 2*</td>
<td>98 ± 4</td>
<td>389 ± 13</td>
<td>12.7 ± 0.9</td>
<td>0.129 ± 0.008</td>
</tr>
<tr>
<td>Fasted post-atropine</td>
<td>8</td>
<td>100 ± 4*</td>
<td>92 ± 5</td>
<td>377 ± 11</td>
<td>11.6 ± 0.8</td>
<td>0.128 ± 0.009</td>
</tr>
<tr>
<td>Hepatic denervation</td>
<td>11</td>
<td>122 ± 4</td>
<td>100 ± 2</td>
<td>401 ± 5</td>
<td>12.6 ± 1.0</td>
<td>0.128 ± 0.011</td>
</tr>
<tr>
<td>Hepatic denervation w/</td>
<td>11</td>
<td>124 ± 5</td>
<td>99 ± 3</td>
<td>396 ± 7</td>
<td>10.8 ± 0.9</td>
<td>0.110 ± 0.010</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial blood pressure; HLBF, hindlimb arterial blood flow; HLVC, hindlimb vascular conductance. Atropine (1 mg/kg) and ACh (2.5 μg·kg⁻¹·min⁻¹) were administered as continuous infusion throughout the test period. *P < 0.01 vs. fed rats.
Protocol 2, vascular response to insulin in fasted rats: effect of atropine. The same experiments described in protocol 1 were performed in rats (n = 8) that had undergone a 24-h fast and no refeed before experiments. This protocol was designed to show lack of HISS metabolic and vascular actions and lack of atropine effects as previously reported (13, 15).

Protocol 3, hepatic denervation on vascular response of insulin, with and without ACh intraportal sustained infusion. Experiments were performed in fed rats with hepatic denervation (n = 11). The RIST, the vascular response to insulin, and the vascular responses to ACh and SNP were conducted before and during the intraportal sustained infusion of ACh at the dose of 2.5 μg·kg⁻¹·min⁻¹. This dose of ACh had previously been demonstrated to be effective to mimic hepatic parasympathetic signals, thus recovering insulin sensitivity (HISS action) following hepatic denervation (36).

Data Collection, Instruments, and Chemicals

Data were acquired digitally and analyzed at a sample rate of 1,000 Hz using IOX data acquisition/analysis system (EMKA Technologies). Blood glucose concentration was measured by a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). The infusion pumps were from Kent Scientific (Torrington, CT; model RS 232). Human biosynthetic insulin was from Novo Nordisk Canada (Mississauga, ON). Atropine, Ach, and SNP were from Sigma. All chemicals were dissolved in saline and prepared daily.

Statistical Analysis

Values are presented as means ± SE. The data were analyzed by paired or unpaired t-test where appropriate. A one-way ANOVA followed by Tukey’s test was employed when the multiple means from different groups were compared. Statistical significance was taken at P < 0.05.

RESULTS

The baseline values from various animal groups with different treatments are presented in Table 1. Compared with normal fed rats, baseline blood glucose concentrations decreased significantly in rats fasted for 24 h. Atropine did not alter HLBF in either fed or fasted rats, indicating absence of a basal cholinergic dilator tone. Hepatic denervation and treatment with atropine or ACh did not alter the baseline glucose level. The baseline arterial blood pressure, heart rate, and arterial HLBF did not show significant variations between the groups, before or after treatment with atropine.

Vascular Responses During the RIST in Normal Fed Rats

Normal fed rats had a RIST index of 315 ± 15 mg/kg. Atropine significantly decreased the RIST index to 148 ± 15 mg/kg, resulting in a 54% inhibition in insulin sensitivity (P < 0.01; Fig. 1A) attributed to HISS action. The MAP and heart rates did not change significantly during the control RIST, but the arterial HLBF and arterial HLVC increased significantly (Fig. 2). Vasodilation was quantified as changes in vascular conductance. Vasodilation was associated with metabolic action reflected by increases in glucose infusion rates that were needed to maintain euglycemia. Vasodilation became statistically significant 10 min after insulin infusion. Both metabolic and vascular indexes followed a pulsatile pattern, rising to a peak at 15–20 min and then returning back to the control level by 35 min (Fig. 2, C and D). Figure 2A illustrates the time course and the pattern of the increases in glucose infusion rate that were needed to maintain euglycemia during the RIST. This pattern was similar to what was observed for the increases in HLVC after insulin administration.

Associated with the decrease in insulin sensitivity, resulting from blockade of HISS release, administration of atropine markedly inhibited the vasodilator response induced by insulin. The maximal increase in vascular conductance following insulin infusion reached ~7% at 10–15 min after atropine treatment, which was significantly less than before atropine treatment (~20%) (Fig. 2D).

Fig. 1. Metabolic responses from the 3 protocols used to manipulate hepatic insulin sensitizing substance (HISS) action. Insulin sensitivity is shown for normal fed rats (A), in rats after a 24-h fast (B), and in rats with hepatic denervation (C). Compared with normal fed rats, the rapid insulin sensitivity test (RIST) index decreased in rats fasted for 24 h. Atropine decreased the RIST index in normal fed rats but had no significant effect on fasted rats in which HISS release was already physiologically suppressed. Hepatic denervation also decreased the RIST index, and intraportal venous infusion (ipv) of ACh restored insulin sensitivity in rats with hepatic denervation. The proportion of the response to insulin administration accounted for by HISS action is shown as %HISS component and is calculated from the difference in the RIST index before and after blockade of HISS release. In C, the HISS component is calculated based on the increased HISS action during ACh infusion. LD, liver denervation. *P < 0.01 vs. corresponding control.
Vascular Responses During the RIST in Rats Fasted for 24 h

Reversible insulin resistance occurs naturally in rats after a 24-h fast as a result of lack of HISS release (15). In the present study, rats with a 24-h fast had a low RIST index of 164 ± 9 mg/kg, which was similar to the index in fed rats after atropine (control RIST index 315 ± 15 mg/kg; after atropine 148 ± 15 mg/kg). Administration of atropine in 24-h fasted rats only slightly further decreased the RIST index to 140 ± 5 mg/kg (Figs. 1B and 3A).

Hindlimb vascular responses following insulin infusion are presented in Fig. 3. With the loss of HISS action in rats fasted for 24 h, infusion of insulin was unable to induce significant vasodilation in hindlimb arterial vasculature. Administration of atropine also had no further significant influence on vascular tone in these rats.

Vascular Responses During the RIST in Rats with Hepatic Denervation

To further determine whether hepatic parasympathetic nerves are involved in the regulation of vasodilation induced by insulin, the rats in this group underwent hepatic denervation. According to the HISS hypothesis, hepatic denervation should prevent the release of HISS in response to insulin. Rats with hepatic denervation showed insulin resistance, as demonstrated by a low RIST index of only 160 ± 6 mg/kg, which represented an ~50% decrease in insulin sensitivity compared with normal rats ($P < 0.01$; Fig. 1C). Associated with the decrease in insulin sensitivity, hepatic denervation also abolished the vasodilation induced by insulin infusion. The increase in HLVC observed in normal fed rats disappeared in the rats with hepatic denervation (Fig. 4D).

Mimicking the hepatic cholinergic signal with intraportal venous infusion of ACh in rats with hepatic denervation restored insulin sensitivity to the level seen in normal fed rats (RIST index 293 ± 13 mg/kg, $P > 0.05$ vs. those from normal fed rats; Figs. 1C and 4A). With restoration of HISS metabolic action, vasodilation to the administered insulin was also restored (Fig. 4).

Correlation of Insulin Sensitivity and Vasorelaxation Induced by Insulin

The relationship between insulin sensitivity (RIST index) and the vasodilation induced by insulin was evaluated by pooling the data from all rats in the three animal groups with different treatments. Vasodilation only occurred in situations where HISS metabolic action was evident, that is, at RIST (100 mU/kg) index levels $>160$ mg/kg (Fig. 5).

Endothelium-Dependent and -Independent Vasorelaxation

ACh and SNP were injected into the lower abdominal aorta. As shown in Fig. 6, ACh at doses of 1 and 3 µg/kg induced increases in vascular conductance by ~110 and 200%, respectively.
tively, in normal fed rats. Hepatic denervation and a 24-h fast did not alter the vasodilation induced by ACh. Atropine, used to block hepatic parasympathetic nerves and therefore HISS release, also completely blocked the direct dilator effect of intra-arterial ACh on the hindlimb.

SNP caused ~90% increase in vascular conductance at 1 μg/kg and ~150% at 3 μg/kg in normal fed rats. Atropine did not have a significant effect on the vasodilation induced by SNP. The same vasodilations to ACh and SNP were observed in rats fasted for 24 h or in fed rats with hepatic denervation with/without ACh intraportal infusion (Fig. 6).

**DISCUSSION**

These studies differentiate metabolic and vascular HISS actions from insulin actions. Elimination of HISS action physiologically (24 h fast), surgically (hepatic denervation in fed rats), and pharmacologically (atropine in fed rats) resulted in reduction or elimination of both metabolic and vascular actions to administered insulin. Restoration of HISS release, using intraportal ACh to mimic the nerve signal, restored both the vascular and metabolic action in rats where HISS release had previously been blocked by hepatic denervation. The direct action of insulin was not associated with vasodilation. Insulin did not cause hindlimb vasodilation in any situation where HISS action was absent, including in fed rats with hepatic denervation or atropine treatment or in normal fasted rats. HISS action accounts for the vasodilator effect seen on insulin administration and is tightly coupled to dynamic HISS metabolic action.

**Confluence of Metabolic and Vascular HISS Action**

In the fed state, the dynamic effect of insulin pulses results in glucose disposal effects due to both the direct action of insulin and the actions of HISS. The HISS-dependent glucose uptake begins ~4 min after the conclusion of the 5-min insulin administration, reaches a peak at ~15–20 min, and returns to baseline by 35 min (15). The HLVC followed a pattern tightly coupled to HISS metabolic action. When HISS metabolic action was absent, as in the situations where it was blocked by atropine (Figs. 1 and 2) or hepatic denervation (Fig. 4), vasodilation also was absent. When insulin was administered to a 24-h fasted rat, where HISS release was physiologically suppressed, no vasodilation was seen, and atropine had no effect on either metabolic or vascular actions of insulin.

When the parasympathetic “feeding signal” in fed rats was eliminated by sectioning the hepatic nerves, the glucose disposal response to insulin was greatly decreased and only the direct effect of insulin remained, with no vasodilation occurring. The permissive parasympathetic feeding signal can be mimicked by continuous intraportal infusion of ACh, as shown by restoration of HISS action in hepatic denervated rats (36) and dogs (21). Here, we confirm that intraportal ACh infusion

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Fig. 3. Changes in glucose infusion rate (A), MAP (B), blood flow (C), and vascular conductance (D) during RIST in rats fasted for 24 h before and after administration of atropine. No HISS action is seen in 24-h fasted rats, and no significant hindlimb vasodilation occurred. The responses are independent of HISS and represent the direct action of insulin. Atropine had no additional effect on the direct metabolic action of insulin and did not alter blood flow.
restores HISS metabolic action and shows for the first time restoration of a tightly coupled vascular response in the hind-limb.

Blood Flow: Cause or Effect

This study shows that the vasodilator effect attributed to insulin is not a direct effect but rather is due to HISS action. The question then becomes whether the regional increase in blood flow contributes significantly to glucose uptake or whether the metabolic action of HISS results in a reactive vasodilation. Unless the blood flow effect is to be attributed with accounting for the majority of HISS metabolic action, the tight coupling of the metabolic and vascular effects is more likely to be explained by reactive dilation. Substantially larger elevations in blood flow have been produced by adenosine (26), bradykinin (28), SNP (27), and IGF-1 (30), with no resultant stimulation of glucose uptake. Vasodilators are not hypoglycemics. It is unlikely that glucose uptake in skeletal muscle is normally limited by the rate of glucose or insulin delivery to tissues. Concentration, rather than bulk delivery, regulates hormonal responses. However, if the skeletal muscle metabolic action of HISS results in reactive hyperemia, the mediators are unknown but could be related to already known mediators of reactive hyperemia including, but certainly not limited to, adenosine, nitric oxide, lactic acid, or other unknown metabolic consequences of HISS action.

No Confluence

The signaling mechanism of insulin in vascular smooth muscle cells has been extensively studied and reviewed (2, 4, 7, 22, 23). However, in none of these studies has HISS action...
been differentiated from insulin action. The ability of insulin to cause HISS release decreases with the duration of fasting so that after an overnight fast much of HISS action has already been suppressed (15, 29). In these conditions, insulin administration would result in minor HISS-related metabolic and vascular action. Some of the controversy as to even the existence of a vasodilator response to insulin may be accounted for by lack of consideration of the prandial status of the experimental subjects.

The physiological and pathological roles of vascular actions of insulin are highly controversial, even as to their existence, but generally are considered to be related to endothelial function (25, 37). Absence of HISS action in the present in vivo studies is not attributable to inhibition of endothelial or smooth muscle cells because the vasodilator responses to both endothelial-dependent (intra-arterial ACh) and endothelial-independent (intra-arterial infusion of SNP) vasodilators were similar in the fed or 24-h fasted state (Fig. 6). Control responses to ACh and SNP were similar in all protocols.

**Hormonal Nature**

HISS action accounts for meal-induced insulin sensitization and for a large proportion of glucose uptake in the fed state. HISS action was first demonstrated based on dynamic metabolic effects (12). This study extends the HISS hypothesis by

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**Fig. 6.** Endothelial-dependent (ACh) and endothelial-independent (sodium nitroprusside; SNP) vasodilation (% change in hindlimb vascular conductance) in normal rats (A) and in rats after a 24-h fast (B) shown before and after atropine administration. The responses of ACh and SNP were not affected by fasting. Atropine blocked HISS release but had no effect on SNP-induced vasodilation, whereas atropine administration blocked both hepatic release of HISS and the direct peripheral action of intra-arterially administered ACh. C: effect of intra-arterial ACh and SNP on hindlimb vasodilation in rats that had undergone hepatic denervation. The vasodilator responses were tested again during continuous infusion of ACh into the portal vein with the intention of restoring HISS release. The peripheral effects of both the endothelial-dependent and endothelial-independent results were unaltered during intraportal ACh infusion. Percent change in vascular conductance = [(B/A) - 1] × 100%, where A represents the basal vascular conductance and B represents the peak vascular conductance after ACh or SNP administration.
confirming the hormonal nature of HISS and demonstrates vascular action as well as the metabolic action of HISS. Hepatic parasympathetic denervation blocked HISS release as shown by both metabolic and vascular responses. Continuous intraportal infusion of ACh mimicked the nerve signal and restored the ability of insulin to stimulate HISS release, increasing both the metabolic and vascular responses. This intraportal dose of ACh is rapidly metabolized and did not recirculate to cause direct effect on the hindlimbs, as baseline glucose and blood flow remained constant. The same dose of ACh given intravenously is rapidly metabolized and did not restore HISS release from the liver and also had no direct effect on the hindlimbs. Intraportal ACh acted on the liver to restore the metabolic response and hindlimb vasodilation. Some substance was released by the liver and has hormonal, metabolic, and vascular effects in the hindlimb.

The AMIS Syndrome

Absence of HISS release requires increased insulin secretion to sequester postprandial glucose. Insulin is lipogenic and chronic absence of meal-induced insulin sensitization (AMIS) results in what we have referred to as the AMIS syndrome (17). The AMIS syndrome begins with chronic absence of HISS release following meals and progresses through a predictable series of homeostatic metabolic dysfunctions, including postprandial hyperglycemia, hyperinsulinemia, hyperlipidemia, increased oxidative stress, and obesity. The impact of the absence of the HISS-dependent vascular response on cardiovascular homeostasis or pathology has not been investigated.

Conclusions

In all three protocols, the vasodilator responses to administered insulin could be attributed to the action of a substance released from the liver and acting peripherally. This hormone has been tentatively named HISS. In conditions where HISS release was blocked (denervation, atropine, fasting), insulin caused no vasodilation. When HISS metabolic action was demonstrated, vascular action was tightly coupled. We conclude that HISS, not insulin, action causes vasodilation to administered insulin. Lack of HISS action has metabolic consequences that lead to pathologies associated with syndrome X (or the metabolic syndrome or cardiometabolic risk or the AMIS syndrome, etc.). The observation that HISS absence also has vascular consequences suggests a further contribution of AMIS to cardiometabolic risk. HISS action on regional vascular beds known to have pathology in diabetes is unknown.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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