Hepatic parasympathetic (HISS) control of insulin sensitivity determined by feeding and fasting

W. W. LAUTT,1 M. P. MACEDO,2 P. SADRI,1 S. TAKAYAMA,3 F. DUARTE RAMOS,2 AND D. J. LEGARE1
1Faculty of Medicine, Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3; 2Institute of Health Sciences, Quinta da Granja- Trav. da Granja, Monte da Caparica 2825, Portugal; and 3Diabetes Center, Tokyo Women’s Medical College, 162-8666 Tokyo, Japan

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WE HAVE RECENTLY DESCRIBED a novel neurohormonal mechanism by which insulin causes release of a putative hepatic insulin sensitizing substance (HISS) from the liver, dependent on a hepatic parasympathetic- and nitric oxide (NO)-mediated permissive reflex (reviewed in Ref. 3). HISS acts on skeletal muscle, but not liver or intestine, to stimulate glucose uptake (17).

Insulin resistance is produced by interruption of the hepatic parasympathetic reflex by surgical denervation of the liver (16, 18), pharmacological blockade of the cholinergic neurotransmitter by using atropine (14–17), or by prevention of hepatic NO liberation by the use of NO synthase antagonists (9, 10). Resistance produced by denervation of the liver can be reversed by intraportal venous, but not central venous, administration of ACh (16) or NO (9). We have suggested that hepatic parasympathetic nerve dysfunction, which causes HISS-dependent insulin resistance (HDIR), may be the cause of insulin resistance in type II diabetes and other conditions, including obesity and chronic liver disease. These studies have recently been reviewed (3). We have further shown that fetal alcohol exposure leads to HDIR in the adult offspring (6).

Insulin sensitivity is assessed by use of the rapid insulin sensitivity test (RIST), which is the acute response to insulin using a rapidly sampled transient euglycemic clamp technique (19). The current operating procedure for this test has been published (4). The RIST index is the amount of glucose required to be administered to maintain arterial euglycemia after a bolus of insulin administered over 5 min. The difference between the RIST index in the control state and after interference with the hepatic parasympathetic nerves reflects the component of insulin action that is dependent on HISS (16). The HISS-independent component of insulin action is quantitated from the RIST index after blockade of HISS release.

Although the chemical identity of HISS remains unknown, the dynamics of HISS action can be further described by examination of the shape of the RIST curve, that is, the progressive rates of glucose infusion required over the test period. In a standard control RIST (insulin dose = 50 mU/kg, male Sprague-Dawley rats), glucose infusion is required to be increased to a maximal level at ~15 min and then decreases, to no longer be required by 35 min.

The objective of the present study was to further define the dynamics of HISS release and the mechanisms controlling this release. We determined the dose-response relationship between insulin and the component of insulin action that is dependent on HISS. Furthermore, we compared the dynamics of HISS release determined by subtracting, from the control RIST curve, the curve obtained after blockade of HISS re-
lease (12). Blockade of HISS release was produced by hepatic surgical denervation, atropine blockade of muscarinic receptors, and blockade of NO production in the liver.

In the original studies showing high variability of the HISS-dependent component of insulin action in ad libitum-fed animals (16), we did not know the duration since the last feeding episode. We have reported, in 1998, in abstract form (5a) and fully report here that the HISS-dependent component of insulin action is maximal after feeding and declines progressively with the duration of fasting. The decrease in insulin action after fasting was shown to be dependent on decreased HISS release. In addition, insulin resistance produced by fasting was partially restored toward the responses seen after feeding by the physical placement of food in the stomach of fasted anesthetized rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (230–300 g) were either fed ad libitum with standard laboratory rat chow (Prolab R-M-H 3000; Agway, Waverly, NY) or were fasted overnight and were offered food for a 1- or 2-h period, as stated, with experiments commencing at 7:00–9:00 AM. There was no tendency for the RIST, expressed per kilogram body weight, to vary with weight (unpublished observation). For the studies related to the effects of fasting, the duration of the fast was timed from the end of the refeeding period. For some series, the rats were tested immediately after the feeding period and are referred to as fed animals. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg). Anesthesia was maintained throughout the experiment by continuous infusion of pentobarbital sodium (1.0 mg/ml saline given at 1.0 ml·100 g body wt⁻¹·h⁻¹) through a cannula in the left jugular vein. Body temperature was monitored with a rectal probe thermometer and was maintained at 37.5 ± 0.5°C by means of a temperature-controlled surgical table and a heat lamp over the table. The rats were heparinized with 100 IU/kg heparin.

Surgical preparation. Spontaneous respiration was allowed through a tracheal tube. Blood samples for glucose analysis (25 μl) were obtained by direct puncture through the silicone tubing of a right femoral arterial-venous loop (19). The right femoral artery was cannulated with the arterial side of the loop. The right femoral vein was cannulated with the venous side of the loop. Arterial blood pressure was monitored via the loop by briefly clamping the silicon sleeve on the venous side. Glucose was infused through the left jugular vein. All other intravenous infusions were given through the venous side of the loop. In experiments in which portal venous drugs were required, the portal vein was cannulated with a 24-gauge (Optiva; Johnson & Johnson Medical, Arlington, TX) intravenous catheter after laparotomy.

The rats were allowed to stabilize from the surgical interventions for at least 30 min before any procedures were carried out. Arterial blood samples were taken every 5 min, and glucose concentrations were immediately analyzed by the oxidase method with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH) until three successive stable glucose concentrations were obtained. The mean of these three concentrations is referred to as the basal glucose level.

RIST. A current operating procedure has previously been described, with discussion of advantages and limitations provided (4, 19). After the basal glucose level was determined, insulin was intravenously infused over 5 min. Unless otherwise stated, the dose of insulin was 50 mU/kg administered in 0.5 ml saline at a rate of 10 mU·kg⁻¹·min⁻¹. Euglycemia was maintained by a variable glucose infusion. The glucose solution was prepared in saline (100 mg/ml) and infused by a variable infusion pump. To avoid hypoglycemia, the glucose infusion (5 mg·kg⁻¹·min⁻¹) was started 1 min after insulin infusion. On the basis of the arterial glucose concentrations measured at 2-min intervals, the infusion rate of the glucose pump was adjusted whenever required to clamp the arterial glucose levels as close to the basal level as possible. After administration of insulin, the rate of glucose infusion required rose to a peak at −15 min and then declined; when no further glucose infusion was required, the test period was concluded, usually within 35 min. The amount of glucose infused after insulin administration represents the magnitude of insulin sensitivity and is referred to as the RIST index.

Effects of denervation, atropine, and NO synthase blockade. These studies were carried out in ad libitum-fed animals. A control RIST index was carried out as described. After the termination of the control RIST, the animals received one of three treatments previously shown to fully block HISS release. One group (n = 10) underwent surgical denervation of the hepatic anterior plexus along the hepatic artery. Another group (n = 8) received an intraperitoneal administration of atropine (3 mg/kg). The third group (n = 18) received an intraperotinal administration of the NO synthase antagonist N-monomethyl-L-arginine (L-NMMA, 0.73 mg/kg). After a new stable baseline had been attained, a second RIST was administered.

The data were analyzed in two ways. First the HISS-dependent component of the control response was calculated by subtraction of the HISS-blocked RIST index from the control RIST index as previously reported (9, 16, 17). A new and more dynamic form of analysis was provided by subtraction of the postmaneuver curve from the control curve throughout the test period. The difference between the two curves provides a plot of the time dependency of the response to HISS.

Insulin and HISS dose-response relationship. All animals in this series had been fasted and refed and were anesthetized for testing immediately on termination of a 1-h refeeding period (n = 13). The objective of this test was to determine the proportion of HISS dependence of insulin action over a range of insulin doses (5–100 μU/kg). Because of technical limitations, it is not possible to obtain the full dose-response data from one animal; therefore, one low and one higher dose of insulin were tested before and after atropine (3 mg/kg iv). Full stabilization was allowed between one higher dose of insulin were tested before and after atropine (3 mg/kg). Further glucose infusion was required, the test period was concluded, usually within 35 min. The amount of glucose infused after insulin administration represents the magnitude of insulin sensitivity and is referred to as the RIST index.

Effect of feeding and fasting on insulin sensitivity. All animals in this group were tested in an identical manner, with establishment of a control RIST followed by a RIST obtained after administration of atropine. One group of rats was tested immediately after the refeed and is referred to as the fed group (n = 6). Three groups of animals were subjected to an 8-h fasting period followed by the 2-h refeed and a subsequent period of timed fasting. One group was tested after 6 h of fasting (n = 6), one after 18 h (n = 6), and another after 24 h (n = 7) from the conclusion of the refeed period.

Effect of food placement in stomach. These animals (n = 7) were subjected to the fast/refeed program and were then
fasted for an 18-h period. A RIST index was obtained, and then a moist mixture of regular rat chow (1 g in 6 ml water) was injected by gavage into the stomach. After reestablishment of a stable glucose baseline, a second RIST was obtained (Fig. 1).

**Drugs and data analysis.** L-NMMA and atropine were purchased from Sigma Chemical (St. Louis, MO). The human insulin was obtained from Eli Lilly (Humulin; Indianapolis, IN). All chemicals were dissolved in saline.

Data were analyzed, when applicable, by using repeated-measures ANOVA followed by the Tukey Kramer multiple-comparison test in each group, one-way ANOVA followed by a Tukey Kramer test, or paired or unpaired Student’s t-tests. The data are expressed as means ± SE throughout. The dynamic RIST curves were analyzed from individual curves with average plots obtained by entering data points at 0.1-min intervals throughout the test. Differences were accepted as statistically significant at P < 0.05. Animals were cared for according to the Guidelines of the Canadian Council on Animal Care, and all protocols were approved by an Ethics Committee on Animal Care at the University of Manitoba.

**RESULTS**

**HISS action revealed by blockade of release.** Control standard RIST indexes (insulin dose = 50 mU/kg) were similar for the groups that received atropine (3 mg/kg iv, n = 8), L-NMMA (0.73 mg/kg iv, n = 18), or surgical denervation of the hepatic anterior plexus (n = 10); the HISS-independent component after these maneuvers was similar. The proportion of insulin action in the control state attributed to HISS was 63.1 ± 6.2% for the atropine group, 58.1 ± 4.7% for the denervation group, and 52.9 ± 3.1% for the L-NMMA group (Fig. 2). The pooled control RIST index (n = 36) was 235.5 ± 7.7, and the RIST index after blockade of HISS release was 100.1 ± 4.7 mg/kg glucose, with the HISS-dependent component of the control RIST accounting for 56.4 ± 2.3% of insulin action. Subtraction of the RIST curve obtained after HISS blockade from the control response revealed the pattern of HISS action, which commenced 3–4 min after the onset of insulin infusion, reached a peak at 15–20 min, and continued for ~9 min after the HISS-independent component of insulin action ceased (Fig. 3). The control RIST no longer required glucose by 31.8 ± 0.9 min, and the HISS-independent insulin action was complete by 22.3 ± 1.9 min. The similarities of response remaining after HISS blockade suggest that denervation, atropine, and NO synthase antagonism had no other effect detected by the RIST than to block HISS-dependent insulin action.

**Variable HISS-dependent component in the ad-libitum-fed group.** In the series shown in Figs. 2 and 3, the animals were fed ad libitum. When the control RIST (HISS-dependent + HISS-independent component) is plotted against the HISS-dependent component (the change in RIST index after HISS release blockade), all three groups show the same relationship (Fig. 4), with a pooled slope of 0.85 ± 0.15 (R² = 0.55, 95% confidence limit 0.55–1.15). The x-intercept (85 mg glucose/kg) is similar to the mean RIST index obtained after HISS blockade (95.4 ± 8.5 mg glucose/kg) shown in Fig. 2. Virtually all of the variability in the control RIST is accounted for by variability in the HISS-dependent component of insulin action as shown by using three means of producing HDIR.

**HISS action is insulin dose dependent.** Rats (n = 13) in this series were refed for 1 h, and the HISS-dependent insulin action was calculated from the difference between the control RIST index and the RIST index obtained after atropine (3 mg/kg iv; Fig. 5). Even though HISS action increased with insulin doses, the proportion of insulin action dependent on HISS was similar (56.5 ± 3.5%) over the full dose range.

The analysis of HISS dynamics for different insulin doses (Fig. 6) showed that the duration of HISS action...
increases with insulin doses (10.9 ± 1.8 min duration for the 5 mU/kg dose up to 31.6 ± 4.9 min for the 100 mU/kg dose). The onset of HISS release occurred at 3–5 min after onset of the insulin administration, independent of the insulin dose. It was also observed that the height and the time to reach the peak of HISS action was insulin dose related.

When two sequential RISTs were performed in the same animal at insulin doses of 10 and 100 mU/kg, a dose-related control RIST index was confirmed (97.0 ± 13.4 vs. 417.8 ± 49.3 mg/kg glucose, P < 0.05, n = 5). The HISS-independent component seen after atropine administration was also significantly dose dependent (31.4 ± 13.4 vs. 139.1 ± 34.0 mg/kg glucose), but the proportion of HISS action remained similar (65.7 ± 5.6 vs. 66.2 ± 6.9%; n = 5).

**Effect of feeding and fasting on insulin and HISS action.** All rats in this series were fasted (overnight) and then offered food for 2 h. The fed group was prepared for testing immediately after the feeding period. Testing times were calculated from the end of the 2-h feed period, and all testing was carried out with surgery commencing between 7:00 and 9:00 AM. Figure 7 shows a progressive decline in control RIST index.
with fasting. The HISS-independent component seen after atropine (3 mg/kg iv) was not significantly altered by fasting. The decrease in insulin action that occurred during fasting was attributed to a decrease in HISS-dependent action.

Effect of food placement in stomach. This group (n = 7) was fasted and refed for 1 h and then fasted for an 18-h period. A RIST index was obtained (126.5 ± 11.3), and then a moist mixture of regular rat chow was injected by gavage into the stomach. The basal control glucose level (105 ± 8 mg/100 g) rose initially after food placement, but by 64 min the level had stabilized at 122 ± 8 mg/100 g. After reestablishment of baseline glucose, a second RIST index was obtained (164.0 ± 12.2, P < 0.001). Placement of food in the stomach was sufficient stimulus to partially reverse fasting-induced HDIR.

DISCUSSION

Whole body glucose uptake in response to a bolus of insulin is severely impaired by surgical or pharmacological interruption of hepatic parasympathetic nerves. The decreased glucose uptake is caused by peripheral insulin resistance and has been attributed to prevention of release of a putative hormone, HISS, from the liver. HISS acts on the hindlimb but does not have significant action on glucose balance across the liver or gut (reviewed in Ref. 3). We report here further studies related to the control of HISS release from the liver in response to insulin administration in anesthetized rats using the RIST (4, 19).

The present data are consistent with the following points. Hepatic surgical denervation, atropine blockade of cholinergic receptors, or blockade of hepatic NO synthase resulted in HDIR through blockade of HISS release. HISS action during the standard (50 mU/kg) RIST was shown to begin after 3–4 min from the onset of insulin action and to continue for 9–10 min after the direct effect of insulin was no longer seen. Calculated from the decline in HISS action from the peak level, the half-life of HISS action is ~9 min. Ad libitum-fed animals showed a range of responses to insulin, as assessed by the RIST; most of the variability was able to be attributed to variability in the HISS-dependent component. In animals in which the time since the last feeding was regulated, the recently fed animals showed a high HISS dependence, with this component decreasing progressively with the duration of fasting. The HISS-independent component was not significantly affected by fasting. The variability of HISS contribution to insulin action in the ad libitum-fed animals may, therefore, be largely due to variability of timing since the last feeding episode. In fed rats, the HISS-dependent component of insulin action was quite constant and accounted for ~55–65% of insulin action in response to a range of insulin doses. Placement of food in the stomach of fasted rats resulted in partial reversal of fasting-induced HDIR.

Technical considerations. The RIST index is the amount of glucose required to be infused to maintain arterial blood euglycemia after bolus insulin administration. At the standard test dose (50 mU/kg), the RIST is complete by 30–35 min and is reproducible up to four times in a row (4). The RIST index showed the same degree of inhibition in response to hepatic denervation (15) as did the insulin tolerance test assessed from the acute hypoglycemia induced by a bolus of insulin (18). In addition, the RIST is validated against the arterial-venous glucose balance measured across the liver, guts, and hindlimb in cats, showing that atropine and surgical denervation cause equal degrees of HDIR but do so through actions on the hindlimb but not liver or gut (17). The present data offer further evidence of the utility of the RIST.

Fig. 4. Control RIST indexes plotted against the HISS-dependent component of insulin action from the groups shown in Fig. 2 using ad libitum-fed rats. Rats showing high insulin sensitivity showed high HISS-dependent insulin action; those showing low insulin sensitivity had low HISS-dependent insulin action (slope 0.85 ± 0.15, R² = 0.59). The intercept at the x-axis (85 mg/kg glucose) is similar to the mean pooled RIST index after HISS blockade shown in Fig. 2 (95.4 ± 8.5 mg/kg). Most of the variability in insulin sensitivity in this ad libitum-fed group was attributable to variability in the HISS-dependent component of insulin action. The correspondence of the data from the three groups supports the conclusion that all three means of reducing insulin action are through the same mechanism with no other significant effects detectable using the RIST.

Fig. 5. Dose-response relationships for HISS-dependent insulin action using the RIST in control state and after HISS action blockade by atropine. The proportion of insulin action accounted for by HISS release in this fed group (n = 13) was 56.5 ± 3.5% and was similar for all doses from 5 to 100 mU/kg. The lack of effect of insulin dose on the proportion of HISS-dependent action was confirmed by paired analysis (n = 5) of dose 10 and 100 in the same animals (see text).
Control RIST indexes reflect the combination of HISS-dependent and HISS-independent components of insulin action. Blockade of HISS release by surgical hepatic denervation, cholinergic muscarinic receptor blockade, or blockade of NO synthase results in a RIST index that is HISS independent. A state of HDIR is seen. The difference between the control RIST index and that after hepatic parasympathetic nerve blockade represents the HISS-dependent component (9, 16, 17). This relationship was evaluated in two distinct ways.

The first method, used in all previous studies, involved simple subtraction of the RIST indexes to determine the proportion of insulin action that was dependent on HISS, whereas the second method involved a temporal evaluation of the RIST curve in which the HISS-independent component was subtracted from the control response to reveal the shape of the curve of the HISS-dependent component of insulin action (12). Mean data from as few as five animals can provide sufficiently smooth curves to permit kinetic determinations.

**HISS pharmacodynamics.** In carrying out the RIST, insulin is administered by intravenous infusion over 5 min, and glucose infusion is commenced after the first minute. Glucose levels are determined at 2-min intervals, and the infusion rate is adjusted to maintain glucose levels at baseline. Glucose infusion is required to be increased to a maximal level at ~15 min, with the insulin dose of 50 mU/kg, and then to decline so that no further infusion is required after 31.2 ± 1.8 min. After denervation or atropine or L-NMMA, the amount of glucose required is substantially reduced (by 56.4 ± 2.3%), with no further glucose required after 22.3 ± 1.9 min. Subtraction of the HISS-independent curve (post-blockade) from the control curve reveals a consistent pattern of response attributed to HISS action. In these standard RISTs (50 mU/kg insulin), HISS action begins ~3–4 min after the onset of insulin action and continues for ~9 min after insulin action has ceased. This may suggest that HISS has an additive rather than a synergistic insulin-like action. Although the
chemical nature of HISS is unknown, the rate of decline of HISS action indicates a half-life of HISS action of ~9 min.

The data shown in Fig. 4 indicate that animals showing a strong reaction to insulin also had a high dependence on HISS, whereas those animals showing a very weak response to insulin showed a small or absent HISS-dependent component. In an attempt to evaluate the reason for the wide variability in the HISS-dependent component, we determined the proportion of insulin action dependent on HISS over a wide physiological range of insulin administration. For this series of studies, fed rats were used. The RIST was determined to be complete when no further glucose administration was required to maintain arterial blood glucose levels steady. This is an important methodological consideration, since the very low doses (5 mU/kg) resulted in a RIST index being obtained within 20 min, whereas the highest doses (100 mU/kg) required 40 min for the response to be completed.

HISS action was insulin dose dependent up to an insulin dose of 100 mU/kg. Doses of 10 and 100 mU/kg insulin resulted in glucose clearance that had a similar proportion of the HISS-dependent component (~66%) at low and high doses when determined in the same animal. This pattern suggests that insulin causes a dose-related secretion of HISS, which accounts for a constant proportion of insulin action over a dose range of 5–100 mU/kg insulin.

From the dynamic HISS curves (Fig. 6), the different doses of insulin caused similar times of onset (3–4 min) and similar rates of rise and decline of HISS action; the dose of insulin mainly affected the time-to-peak HISS action and the magnitude of the peak and, in this manner, determined the total HISS response and duration of action. HISS action continued for longer than the HISS-independent component at all doses. Attempts to use doses of 200 and 300 mU/kg insulin resulted in responses in which the ratios of HISS-dependent and -independent action were not consistent within the group and suggested that high acute doses of insulin often resulted in a decrease in the HISS-dependent component. These data are not reported (M. P. Macedo and D. Ramos, unpublished observations) and require further study.

Effect of feeding on HISS release. Although the original studies showing high HISS-dependent variability in insulin action were obtained in animals that had ad libitum access to food, the duration since the last feeding was unknown. To control the duration of fasting more precisely, rats were fasted overnight and then offered food for a 2-h period. The fasting time was taken as the time from the end of the 2-h refeeding period.

Animals tested immediately after the feeding period showed a high responsiveness to insulin and a high HISS dependence, whereas increasing periods of fasting led to a decrease in insulin action, accounted for by a decrease in the HISS-dependent component. The HISS-independent component was similar in rats that had been recently fed or that had been subjected to a 6-, 18-, or 24-h fast. Thus the reason for some of the variability in the ad libitum-fed groups may be due to variability in timing since the last feeding or, at least, in some signal related to feeding.

Because of the strong dependence of HISS action on the prandial status, we now routinely carry out the fast and refeding protocol and test animals in the fed state for most mechanistic studies. Although not originally encountered, subsequent experiments have indicated that some of these animals, even though fasted overnight, do not eat during a 1-h period, as demonstrated by lack of food in the stomach and high insulin resistance. We now routinely verify that these animals have eaten by inspecting for food content in the stomach. It is also our impression that allowing a 2-h refeed results in a higher incidence of feeding. All fed animals were allowed the 2-h refeed except for those in the dose-response series.

To further investigate the effect of feeding on the release of HISS, rats were fasted, refed, and fasted for 18 h. A RIST was determined, and then rat chow was moistened and injected by gavage in the stomach of the anesthetized rat. After glucose levels restabilized (~60 min), the RIST was repeated. The procedure resulted in the RIST index increasing toward levels seen in fed animals, thus indicating that food in the stomach was adequate stimulus to signal the fed state. At this point, we have not determined what the signal from the gut is that indicates that the animal is in the absorptive postprandial state. Amino acids in the portal blood have been shown to activate hepatic vagal afferent nerves (13). Intraperitoneal administration of a mixture of gluconeogenic amino acids tended to direct glucose carbon away from the liver during the administration of glucose, with insulin and glucagon held steady by using the euglycemic clamp technique (7). This diversion of glucose uptake to extrahepatic sites is consistent with an effect mediated by HISS, which is shown to cause increased glucose uptake across the hindlimb but not in the liver or gut (17). Nutrients and hormones in portal blood, including glucose, amino acids, glucagon, CCK, somatostatin, leptin, and interleukins, and changes in osmotic pressure are also known to activate receptors in the liver, leading to altered hepatic vagal afferent nerve discharge (8). We have not evaluated these and other possible mediators of the prandial signal.

Whatever the prandial signal is, it is well maintained in the anesthetized animal since no decrease in the RIST index was seen in rats that were administered four consecutive control RISTs (4) over about a 6-h period (up to 6 repetitions have now been done over ~8 h; P. Sadri and D. J. Legare, unpublished observations). In contrast, rats fasted for 6 h show a significant decline in the RIST index (Fig. 7). This anomaly may be related to the delayed gastric emptying seen in the anesthetized rat where, by qualitative visual assessment, the volume of food content in the stomach does not change notably over a 6- to 8-h experiment.
Physiological regulation of HISS-dependent insulin action. Regulated production of HDIR in the fasted state would confer a protective role, minimizing the hypoglycemic effect of insulin in the absence of ingested glucose. Insulin secretion shows pulsatile or rhythmic secretion in fasted rats independent of arterial glucose (2), and only about one-half of the daily insulin secretion is meal related (1). Conditioned reflex secretion of insulin is also able to reach early peaks at similar levels to those produced by a meal, even in the absence of a meal (11). In such situations, it is not useful for insulin to exhibit full action since the result could be hypoglycemia. HISS, therefore, provides a useful physiological mechanism to selectively partition glucose to skeletal muscle when glucose absorption is high but would minimize uptake by muscle in the fasted state or in any other state of HDIR.

The hepatic parasympathetic nerves appear to play a permissive regulatory role in that, when neural input is high, insulin is able to release HISS. The permissive nature can be shown by the experiment of producing HDIR by denervating the liver and then restoring the signal by continuous intraportal infusion of ACh or the NO donor 3-morpholinosydnonimine. Baseline glucose disposal is not notably altered by the drugs before administration of the bolus of insulin, but the HISS release in response to insulin, administered 15–30 min after the onset of drug infusion, is restored (9, 16).

In response to insulin administration, a permissive hepatic parasympathetic reflex, mediated by cholinergic muscarinic receptors and NO, leads to secretion of a hepatic insulin-sensitizing substance that accounts for ~60% of the glucose uptake response to a bolus of a wide range of doses of insulin. The mechanism by which HISS operates is unclear and could either represent a potentiation of insulin action or an additive effect attributable to HISS directly. Because of the longer duration of HISS-dependent action compared with HISS-independent insulin action, the latter interpretation seems most likely. The HISS release in response to insulin is controlled by the prandial status of the animal so that animals that are recently fed show ~60% of insulin action to be HISS dependent; the HISS-dependent component decreases with the duration since last feeding, whereas the HISS-independent action is not affected by prandial status. The signal indicating prandial status is unknown, but placement of food in the stomach of the anesthetized rat is able to serve as a prandial signal to result in increased HISS release in response to insulin. Additional studies are required to delineate the pathways of regulation of HISS release and the chemical nature of HISS.

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