Insulin sensitivity regulated by feeding in the conscious unrestrained rat

Martin G. Latour and W. Wayne Lautt

Abstract: Hepatic insulin sensitizing substance (HISS), a putative hormone released from the liver in response to insulin in fed animals, accounts for 50–60% of insulin action. HISS release is regulated by permissive control of the hepatic parasympathetic nerves. The objectives were to develop the rapid insulin sensitivity test (RIST) in conscious rats, and to assess the effects of anesthesia, atropine, feeding, and fasting on insulin action. The RIST index, expressed as milligrams glucose per kilogram body weight required to maintain euglycemia after a 50 mU/kg bolus of insulin, was similar in conscious and anesthetized rats (238.6 ± 42.5 vs. 225.3 ± 30.4 mg/kg). Atropine produced a 56% inhibition of insulin action in fed rats. After a 24 h fast, full HISS-dependent insulin resistance had developed as shown by a low RIST index that was not reduced further by atropine. Fasting caused a 10.5% decrease in insulin action per hour over six hours. HISS-dependent insulin resistance in 24-h fasted rats was reversed 4 h after re-feeding (90.9 ± 12.3 vs. 204.5 ± 30.5 mg/kg). We conclude that HISS-dependent and HISS-independent insulin action, as assessed by the RIST, is similar in conscious and pentobarbital-anesthetized rats. Pharmacological blockade of HISS-dependent insulin action and physiological regulation of HISS action by feeding–fasting is confirmed. Re-feeding fasted rats reversed HISS-dependent insulin resistance. Merits of use of the RIST in conscious versus anesthetized rats are discussed.

Key words: insulin action, insulin resistance, anesthetic effect, glucose, fasting, HISS.

Introduction

We have recently described a novel neurohumoral mechanism by which hepatic parasympathetic nerves, through permissive release of a putative hepatic insulin sensitizing substance (HISS), regulate the glucose disposal resulting from a bolus injection of insulin. Fasting results in a progressive decrease in HISS release so that the HISS-dependent component of insulin action is minor or insignificant by 24 h (Lautt et al. 2001). All of these and related studies that have been recently reviewed (Lautt 1999) were carried out in cats or rats that had been anesthetized with pentobarbital and utilized the rapid insulin sensitivity test (RIST) (Lautt et al. 1998; Xie et al. 1996) to assess insulin sensitivity. The RIST index is the amount of glucose required to be administered to maintain the rapid insulin sensitivity test (RIST) (Lautt et al. 1998; Xie et al. 1996) to assess insulin sensitivity. The RIST index is the amount of glucose required to be administered to maintain the postprandial state, hepatic parasympathetic nerves allow a pulse of insulin to cause the secretion of HISS from the liver; HISS stimulates peripheral glucose uptake and accounts for 50–60% of the glucose disposal that is seen following a bolus injection of insulin. Fasting results in a progressive decrease in HISS release so that the HISS-dependent component of insulin action is minor or insignificant by 24 h (Lautt et al. 2001). All of these and related studies that have been recently reviewed (Lautt 1999) were carried out in cats or rats that had been anesthetized with pentobarbital and utilized the rapid insulin sensitivity test (RIST) (Lautt et al. 1998; Xie et al. 1996) to assess insulin sensitivity. The RIST index is the amount of glucose required to be administered to maintain the postprandial state, hepatic parasympathetic nerves allow a pulse of insulin to cause the secretion of HISS from the liver; HISS stimulates peripheral glucose uptake and accounts for 50–60% of the glucose disposal that is seen following a bolus injection of insulin. Fasting results in a progressive decrease in HISS release so that the HISS-dependent component of insulin action is minor or insignificant by 24 h (Lautt et al. 2001). All of these and related studies that have been recently reviewed (Lautt 1999) were carried out in cats or rats that had been anesthetized with pentobarbital and utilized the rapid insulin sensitivity test (RIST) (Lautt et al. 1998; Xie et al. 1996) to assess insulin sensitivity. The RIST index is the amount of glucose required to be administered to maintain the postprandial state, hepatic parasympathetic nerves allow a pulse of insulin to cause the secretion of HISS from the liver; HISS stimulates peripheral glucose uptake and accounts for 50–60% of the glucose disposal that is seen following a bolus injection of insulin. Fasting results in a progressive decrease in HISS release so that the HISS-dependent component of insulin action is minor or insignificant by 24 h (Lautt et al. 2001). All of these and related studies that have been recently reviewed (Lautt 1999) were carried out in cats or rats that had been anesthetized with pentobarbital and utilized the rapid insulin sensitivity test (RIST) (Lautt et al. 1998; Xie et al. 1996) to assess insulin sensitivity. The RIST index is the amount of glucose required to be administered to maintain the postprandial state, hepatic parasympathetic nerves allow a pulse of insulin to cause the secretion of HISS from the liver; HISS stimulates peripheral glucose uptake and accounts for 50–60% of the glucose disposal that is seen following a bolus injection of insulin. Fasting results in a progressive decrease in HISS release so that the HISS-dependent component of insulin action is minor or insignificant by 24 h (Lautt et al. 2001). All of these and related studies that have been recently reviewed (Lautt 1999) were carried out in cats or rats that had been anesthetized with pentobarbital and utilized the rapid insulin sensitivity test (RIST) (Lautt et al. 1998; Xie et al. 1996) to assess insulin sensitivity. The RIST index is the amount of glucose required to be administered to maintain


M.G. Latour and W.W. Lautt.1 Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, MB R3E 0W3, Canada.

1Corresponding author (e-mail: wlautt@cc.umanitoba.ca).
a euglycemic baseline following a bolus (5 min) administration of insulin (50 mU/kg).

Glucose disposal following an injection of insulin consists of a HISS-independent component and a HISS-dependent component. Blockade of HISS release can be produced by surgical denervation of the liver (Xie and Lautt 1996a, 1996b), atropine blockade of hepatic muscarinic receptors (Xie and Lautt 1995, 1996a), or blockade of hepatic nitric oxide synthase production (Sadri and Lautt 1999). All of these maneuvers result in the RIST index decreasing by 50–60% and result in a condition referred to as HISS-dependent insulin resistance (HDIR). HDIR is also regulated physiologically in response to feeding and fasting (Lautt et al. 2001). Although the HISS story to date (reviewed by Lautt 1999) has relied primarily upon data generated using the RIST in anesthetized animals, some protocols are clearly best carried out in conscious unrestrained animals. The primary objectives of this study were (i) to establish the RIST in a conscious rat model; (ii) to determine the impact of anesthesia on the RIST; (iii) to determine if the HISS-dependent component of insulin action could be eliminated using pharmacological blockade; and (iv) to determine if HISS-dependent insulin action is physiologically regulated by the feed–fast status in conscious animals as it had been previously shown in the anesthetized condition.

The results demonstrated a remarkable similarity in quantification of insulin-induced glucose disposal using the recently described RIST and confirm that pharmacological manipulation and physiological regulation of the HISS-dependent and HISS-independent components of insulin action are similar in pentobarbital-anesthetized or conscious rats. We show, for the first time, that the HDIR (physiologically produced by fasting) is reversed by feeding in the conscious unrestrained rat.

Materials and methods

We recently described and evaluated a RIST to assess insulin action in vivo in anesthetized cats and rats (Xie et al. 1996). Several years of experience with the RIST led to further modifications and accumulation of technical information (Lautt et al. 1998). In this report we provide an adaptation of the method in the conscious unrestrained rat.

Animal care

Male Sprague–Dawley rats (weighing 220 ± 10 g) were allowed ad libitum access to standard rat chow (Prolab R-M-H 3000 Agway, Waverley, N.Y.) and tap water and were subjected to a controlled environment (lights on 0600–1800; room temperature 20°C) for at least five days before surgery. All experiments began between 0700 and 0900. Animals in the fed groups were fasted for 24 h, insulin sensitivity (the RIST) was assessed, and the rats were allowed food for 2 h prior to retesting. All protocols were conducted according to the directives of the Canadian Council on Animal Care and were approved by the Ethics Committee of Animal Care at the University of Manitoba.

Surgical preparation

Chronic implantation of jugular venous and carotid arterial catheters was carried out under anesthesia with isoflurane (1–3% inspired; AErrane). All instruments were washed with a solution of Dettol (3% in a 70% ethanol solution) and the skin area was cleansed with a solution of 2% Hibitane. The implantation of the arterial and venous catheters was performed 4–7 days prior to the experiment using Micro-Renathane® 40 catheters (Braintree Scientific, Braintree, Mass.). The catheters were advanced toward the heart between 2.5 (carotid) and 3.5 cm (jugular) from the entry point and were tied and glued into place. All catheters were subcutaneously tunneled and glued behind the shoulder blades of the animal. They were subsequently filled with a solution of sodium heparin (200 UI/mL) and closed with a blunted and crushed needle. At the end of the surgery, all skin openings were closed with silk sutures. On several occasions, various combinations of tubing were used for the carotid artery and jugular vein catheters (polyethylene and Micro-Renathane® tubing from Intramedics Clay-Adams: PE 50–PE 50, PE 50–PE 60, MRE 40–MRE 40, MRE 40–MRE 50). None of these combinations produced qualitatively different responses, thus allowing operator preference to determine the material constituent of the loop.

Arterial–venous sampling loop

On the morning of the experiment all rats were weighed and brought to the laboratory, allowing them to adapt to the new environment. The carotid arterial and jugular venous catheters were connected to a silicone arterio-venous (AV) loop prefilled with saline–heparin (200 IU/mL) to establish a continuous blood flow through the vascular shunt. The sampling loop has been described in the anesthetized rat (Lautt et al. 1998). The removal of blood clots within the chronically implanted catheters was achieved with a prefilled saline–heparin 1 cc syringe and a blunted 21 gauge 1 in. needle. Both ends of the AV silicone loop were connected with a 5 mm tygon spacer that fitted the outer bore of a 7 cm long PE 60 tube, and a 10 mm cut 21 gauge blunted needle was inserted at the end. During the connecting process, the venous side of the loop was clamped by a vascular hemostat. Once the arterial flow was established, the silicone sleeve of the arterial side of the loop was clamped and the venous side was unclamped. The right jugular vein catheter was then cleared of clots and connected to the venous side of the AV loop. The clamp on the arterial side was opened, allowing blood to flow through the loop. Arterial blood continuously flowed through the circuit into the venous side. With some rats, this maneuver required the use of a restrainer bag (DecapitCones, DC-200, Braintree Scientific, Braintree, Mass.), which was removed once the AV loop was connected. The AV shunt was modified from the previously described loop (Lautt et al. 1998) by inserting a cut blunted needle at each end from the time of surgical implantation to the establishment of the functional shunt.

By use of the AV shunt, arterial blood samples (25 μL) were taken directly from a moving stream of blood with no need to wash or flush sampling catheters. The infusion catheters were made of PE 50 tubing with a cut and slightly bent 23 gauge needle at the delivery end of the catheter. Insulin,
glucose, and atropine were administered intravenously by puncturing the sleeve on the venous side of the loop. Arterial blood pressure, measured via the side branch of the loop, was determined by a brief occlusion of the venous outlet upstream from the infusion lines. Recording of non-occluded loop pressure is useful to continuously assess the patency of the loop. Clotting can occur in either the arterial or venous end of the shunt during long experiments. This situation may occur in experiments even where supplemental heparin is administered. The pressure monitoring allows early identification of clotting, with the monitored pressure rising if the clot is at the venous outlet of the loop and the pressure dropping if the clot is at the arterial inlet side. To verify and visualize the flow in the shunt, it is necessary to inject a small quantity of saline into the silicone sleeve. Upon identification of a problem with loop patency, the side branch can be used to flush the catheters or to remove clots after occluding the appropriate limb of the shunt.

**RIST in the conscious rat**

Following the connection of the AV shunt, the animal was allowed to stabilize for a minimum of 15 min before any blood samples were drawn. Following the stabilization period, the baseline glucose levels were determined by samples taken at 5 min intervals until three successive stable determinations were made. The mean of these 3 data points was used as the ideal euglycemic baseline to be maintained during the euglycemic clamp. Insulin infusion was commenced using an infusion pump (Harvard Apparatus, Saint-Laurent, Que.) to administer the dose of 50 mU/kg over 5 min. After 1 min of insulin infusion, the first test glucose sample was determined and glucose infusion (n-glucose–saline solution; 100 mg/mL) was started at a rate of 5 mg·kg⁻¹·min⁻¹. Arterial glucose levels were sampled at 2-min intervals throughout the test period, with glucose infusion rates adjusted to maintain euglycemia. Typically, using the standard insulin dose (50 mU/kg), the glucose infusion required rose to 10–15 mg·kg⁻¹·min⁻¹ and peaked at about 15 min and then declined to zero by 35 min. The RIST index is the amount of glucose (mg/kg body weight) required to be infused over the test period to maintain euglycemia.

Blood glucose was analyzed by the oxidase method with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Although deviation from the ideal glycemia was rapidly correctable because of the 2 min feedback, any deviation of more than 10% resulted in the rejection of the RIST. The average percent deviation from the target glycemic level for each RIST was 1.5 ± 0.5%.

To assess the effects of anesthesia, a RIST was conducted in the conscious state and following anesthesia. In the anesthetized group, the acute implantation of the carotid and jugular catheters was conducted after sodium pentobarbital anesthesia (50 mg/kg, i.p.) which was further maintained by continuously infusing diluted sodium pentobarbital (10 mg·kg⁻¹·h⁻¹, i.v.) in saline. An unpaired design was selected because of the previous observation that fasting resulted in an approximate 10% reduction in RIST index per hour of fasting. Both groups were tested within 1 h of cessation of the re-feeding period.

Results are expressed as means ± SE. Statistical analyses were made using paired t tests or, when applicable, repeated measure ANOVA followed by Tukey’s honestly significant difference. Data compared followed a normal distribution. Differences were accepted as statistically significant at $P < 0.05$.

**Results**

**Consecutive RISTs in the conscious fed rats**

The body weight ($n = 6$) was fully recovered from the day of surgery (291 ± 7 g) to the day of the experiment (302 ± 4 g). The time between each RIST was between 30 and 45 min with all four tests being completed within 6 h. The insulin sensitivity, as assessed by the RIST index (milligrams glucose per kilogram body weight infused during the test to maintain euglycemia), progressively decreased (first vs. fourth RIST, $P < 0.01$; 228.1 ± 21.6 vs. 135.0 ± 14.1 mg/kg; Fig. 1). The average decrease in the RIST index was 10.5 ± 3.7% per hour. Prior to each RIST, basal glucose levels (mg/dL) were re-stabilized (115.8 ± 4.3; 113.6 ± 4.2; 116.4 ± 3.3; 113.7 ± 3.1; not significantly different). The mean arterial pressure (mmHg) did not significantly change (115 ± 7; 114 ± 5; 99 ± 8). The hematocrit decreased slightly by the final test ($P < 0.05$; 43 ± 1% vs. 40 ± 3%).

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Reversal of fasting-induced HDIR by feeding

Rats (n = 5; 279 ± 6 g) were fasted for 24 h, administered a RIST, fed, and retested after a new glycemic baseline had stabilized after 4 h (3 h 57 ± 9 min). Feeding resulted in an increased basal glucose level (P < 0.01; 87.8 ± 2.8 vs. 120.2 ± 2.0) and an increased RIST (90.9 ± 12.3 vs. 204.0 ± 25.0 mg/kg) (Fig. 2).

HDIR produced by fasting or atropine

The RIST obtained in 3 groups of rats, fed (n = 5), fasted for 24 h (n = 5), and fed but treated with atropine (3 mg/kg), is shown in Fig. 3. The degree of HDIR induced by fasting and pharmacological blockade of HISS release was similar. The HISS-independent component of insulin action remaining was similar after physiological (fasting) or pharmacological (atropine) blockade of HISS-dependent insulin action.

Effects of anesthesia

An unpaired comparison (Fig. 4) between the RIST index in conscious rats determined within 1 h after the end of the feeding period and the RIST index in rats administered anesthesia within 1 h post-feeding showed similar RIST indexes.

Discussion

The concept and quantification of HISS-dependent and HISS-independent insulin action, previously studied only in animals anesthetized with pentobarbital, has been confirmed in the conscious unrestrained rat using the RIST. Pentobarbital anesthesia did not alter the RIST index. Fasting resulted in a progressive decrease in HISS-dependent insulin action so that, by 24 h, insulin action was independent of HISS and a condition of HISS-dependent insulin resistance (HDIR) occurred. The HDIR seen in a 24-h fasted state was reversed by feeding.

Effect of fasting on the RIST

We have previously reported that 4–6 consecutive RISTs can be carried out in the anesthetized rat with no significant change in insulin sensitivity detected over the 5–8 h period of testing (Lautt et al. 1998). A similar time-control study reported here for conscious rats indicates a normal first RIST index (228.1 ± 21.6 mg/kg) that declined progressively and significantly to 135.0 ± 14.1 mg/kg by the fourth test. The progressive decrease in insulin sensitivity could suggest a test-induced artifact. An alternative explanation, which we propose, is that the progressive decline in RIST index is related to the time since feeding. This is supported by a number of lines of evidence.

In a recent study, we reported the physiological regulation of the HISS-dependent component of insulin action by the prandial condition of the rat (Lautt et al. 2001). Rats were fasted and then re-fed, as in the present study, to tightly regulate the feeding time, and then were tested under anesthesia after varying periods of fasting. A control RIST index, similar to that reported in the immediate postprandial RIST in the conscious animals, decreased in animals tested 6 h after the completion of the feeding period. The decrease from 212 to 150 mg/kg at 6 h represented a 10.3% decrease per hour of fasting in the anesthetized animals. This is similar to the 10.5% decrease per hour in RIST index that progressively developed up to the 6 h postprandial time in the conscious animals. Further, rats tested within 1–1.5 h after feeding showed similar insulin sensitivity based on the RIST index in the conscious and anesthetized state (Fig. 4).

Although the specific characteristics of the feeding signal have not been defined, comparison of the time controls in the conscious versus anesthetized animals may provide some clues. The effect of fasting can be equally clearly demonstrated in unpaired anesthetized animals tested after various periods of fasting or tested sequentially in a paired design in the same conscious animals. An apparent anomaly is seen, however, with the surprisingly well maintained insulin sensitivity determined in 4–6 sequential tests in an anesthetized animal, contrasting with the progressive decline in the conscious rats. We have noted that the food content of the stomach of the anesthetized animals does not appear to vary over an entire 8 h experiment. The effect of anesthesia to paralyze gastric motility and delay gastric emptying would seem to be the reason for the well maintained RIST index in the anesthetized animal in contrast to the progressively decreasing RIST index.
that is seen in conscious animals in response to the duration of fasting. Thus the prandial signal does not change after the induction of anesthesia so that fasting in the anesthetized state does not lead to a progressive HDIR. The effect of fasting on HISS action is clearly demonstrated by the production of severe HDIR as a result of a 24-h fast. These rats were then allowed a period of re-feeding and were tested 4 h after the feeding episode. The fasting RIST index (90.9 ± 12.3 mg/kg) was restored to a normal level (for a 4 h postprandial state) following re-feeding (204.0 ± 25.0 mg/kg). The physiological regulation of HISS-dependent insulin action was confirmed by the reversal of fasting-induced HDIR by feeding in conscious unrestrained rats. These data from conscious rats also confirm that complete HDIR can be produced by a 24-h fast or blockade of HISS release using atropine in fed rats.

Practical use of the RIST

The RIST can clearly be used to quantitate the glucose disposal effect of a bolus of insulin equally well in conscious or pentobarbital-anesthetized rats. The strong similarity of pharmacological blockade of HISS-dependent insulin action and physiological regulation by the feed–fast process indicates that in many experimental protocols the additional time requirement for post-implantation recovery and the additional intrinsic difficulties with conscious animal studies need not be undertaken.

Some protocols (e.g., the reversal of fasting-induced HDIR by re-feeding) can best be done using the conscious preparation. However, the conscious studies have one very clear disadvantage. Time controls, so essential to paired studies, are more complex in the conscious rat where gastric paralysis does not occur and a progressive decline in insulin sensitivity is seen with the duration since last feeding. Although the insulin sensitivity decrease determined by the RIST only became significant after the fourth test, a consistent time-dependent decrease of 10.5 ± 3.7% per hour post-feeding is seen throughout the test period. Thus, for studies requiring a constant background of insulin sensitivity upon which to assess pharmacological or other interventions, the anesthetized preparation would clearly be preferable.

Conclusions

The RIST is a powerful research tool to assess the glucose disposal action of an injection of insulin in both conscious and pentobarbital-anesthetized rats. The RIST index is not significantly affected by pentobarbital anesthesia. The progressive effect of fasting to decrease the HISS-dependent component of insulin action is demonstrated in both anesthetized and conscious preparations; however, the progressive decline in the RIST index induced by fasting is halted by anesthesia so that the time under anesthesia is not considered additional fasting time. The reversal of HISS-dependent insulin resistance caused by fasting was demonstrated by the provision of food to a conscious rat tested before and after feeding. These studies validate the use of the RIST for determination of HISS-dependent and HISS-independent components of insulin action and provide guidelines for the selection of the conscious versus anesthetized preparations.

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