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Meal-induced enhancement in insulin sensitivity is not triggered by hyperinsulinemia in rats

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Abstract

Several reports confirmed the phenomenon of postprandial increase in whole-body insulin sensitivity. Although the initial step of this process is unknown, the pivotal role of postprandial hyperinsulinemia has strongly been suggested. The aim of the present study was to determine whether hyperinsulinemia per se induces insulin sensitization in healthy male Wistar rats. Rapid insulin sensitivity test (RIST) were performed in fasted, anesthetized rats before and during stable hyperinsulinemia achieved by hyperinsulinemic euglycemic glucose clamping (HEGC) with insulin infused either through the jugular vein (systemic HEGC) or into the portal circulation (portal HEGC) at a rate of 3 mU/(kg min). Insulin sensitivity expressed by the rapid insulin sensitivity (RIST) index (in milligrams per kilogram) was characterized by the total amount of glucose needed to maintain prestudy blood glucose level succeeding an intravenous bolus infusion of 50 mU/kg insulin over 5 minutes. In fasted animals, the RIST index was 37.4 ± 3.1 mg/kg. When hyperinsulinemia mimicking the postprandial state was achieved by systemic HEGC, the RIST index (39.7 ± 10.6 mg/kg) showed no significant changes as compared with the pre-HEGC values. Hyperinsulinemia achieved by portal insulin infusion also failed to modify the RIST index (35.7 ± 4.3 mg/kg). The results demonstrate that acute hyperinsulinemia, no matter how induced, does not yield any sensitization to the hypoglycemic effect of insulin.

1. Introduction

Recently, a novel endogenous insulin-sensitizer mechanism was described by Lautt [1] termed as the *hepatic insulin-sensitizing* (*HISS*) mechanism. According to the original hypothesis, the mechanism was regulated by feeding [2], with maximum insulin-sensitizing effect seen after a meal and with a decrement until the next meal. The phenomenon was proposed to be underpinned by postprandial activation of the anterior hepatic plexus (AHP) fibers that resulted in the release of a currently undefined substance termed *HISS* from the liver, which, upon entering the circulation, sensitizes peripheral tissues (predominantly the skeletal muscle and adipose tissue) to the hypoglycemic effect of insulin [1]. Extensive studies on the HISS mechanism revealed that both partial hepatic denervation [3] and pharmacologic blockade of either neural nitric oxide synthase [4] or hepatic muscarinic receptors [3] inhibited meal-induced insulin sensitization. Porszasz et al [5] suggested that nerve fibers within the AHP responsible for this phenomenon are of a sensory nature, belonging to a population of capsaicin-sensitive primary afferent fibers of the vagus nerve. This derived from the observation that perineural treatment of the AHP with 2% capsaicin solution resulted in neurogenic insulin resistance [5]. An accidental finding that the phenomenon of meal-induced insulin sensitization was impossible to reproduce in rabbits when anesthesia was achieved by drug combinations containing diazepam, a prototype of benzodiazepine sedative hypnotics with cholecystokinin (CCK) receptor antagonistic effect [6], shed light on the involvement of CCK in HISS-dependent insulin sensitization [7]. Nevertheless, Lautt's [8] original suggestion that the HISS-dependent pathway is essentially activated by the postprandial release of insulin has never received a strong experimental support.

The aim of the present study was therefore to investigate whether postprandial hyperinsulinemia was the principal cause of activation of the HISS mechanism as suggested by

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Lautt [8] or whether it can better be attributed to a series of events that follow food intake. As a methodological approach, whole-body insulin sensitivity was determined by means of the rapid insulin sensitivity test (RIST) in fasted Wistar rats, a method frequently used by Lautt, in the presence or absence of controlled hyperinsulinemia attained by hyperinsulinemic euglycemic glucose clamping (HEGC) method [9].

2. Methods

2.1. Ethics

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals and conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (publication no. 85-23, revised 1996). The experimental protocol applied has been approved by the local ethical boards of the University of Debrecen, Hungary (DEMAB 034/2005).

2.2. General procedures

Male Wistar rats weighing 300 to 350 g (purchased from Charles River Laboratories, Budapest, Hungary) were used throughout the experiments. The animals—fasted overnight—were anesthetized with intraperitoneal injection of thiopental sodium (50 mg/kg). The trachea was cannulated, and the animals were allowed to breath freely through the cannula. Continuous anesthesia was maintained by succeeding intravenous infusion of thiopental sodium solution of 1.5 mg/100 g body weight through a catheter inserted into the right femoral vein. For continuous infusion necessary to the HEGC procedure, polyethylene catheters connected to infusion pumps (Syringe Pump 11 Plus; Harvard Apparatus, Holliston, MA) were introduced into 2 branches of the left jugular vein for insulin and glucose infusion, respectively. The left femoral vein was cannulated for bolus insulin and subsequent glucose administration during the RIST. The left carotid artery was also cannulated for arterial blood sampling and for measurement of mean arterial pressure by means of a Statham P23 DB transducers attached to an electromanometer (Experimetria, Budapest, Hungary). To prevent blood coagulation, a bolus of heparin (100 IU/kg) was given intravenously. The body temperature was kept constant at 37°C ± 0.5°C by means of heating operating table (Type 872H with heating controller Type 861; Hugo Sachs Elektronik–Harvard Apparatus, March-Hugstetten, Germany). At the end of the experiments, the animals were killed by an intravenous overdose of thiopental sodium (100 mg/kg).

2.3. Rapid insulin sensitivity test

The RIST was preformed with 50 mU/kg insulin as described by Lautt et al [10]. In brief, after a 30-minute postsurgery stabilizing period, arterial blood samples were taken every 5 minutes for blood glucose determination. The mean blood glucose level of 3 consecutive determinations was referred to as the *control value*. The total amount of glucose (expressed in milligrams per kilogram of body weight) required to counteract the hypoglycemic effect of 50 mU/kg insulin infusion and to maintain the control blood glucose level produced the RIST index as an indicator of whole-body insulin sensitivity [10,11].

2.4. Hyperinsulinemic euglycemic glucose clamp

The animals were prepared as described above. The hyperinsulinemic state was achieved by 2 different ways of insulin infusion. In the first case (systemic HEGC), insulin was infused directly into the systemic circulation through the jugular vein prepared at a rate of 3 mU/(kg min). In the second case (portal HEGC), insulin was infused into the spleen vein at the same rate to mimic physiologic entry of pancreatic insulin into the circulation through the portal vein and the liver. Beyond the difference in route of insulin administration, the 2 protocols did not differ from each other; the glucose infusion was administered into a peripheral vein to compensate for the hypoglycemic effect of hyperinsulinemia.

Human regular insulin (Humulin R; Eli Lilly, Indianapolis, IN) was infused continuously through either the systemic or the portal infusion line, and the glucose infusion (20% wt/vol) rate was adjusted to maintain the target blood glucose level (5.5 ± 0.5 mmol/L in the fasted state determined before the procedure as the control value). Blood samples (0.1 mL) were obtained from the carotid artery for determination of blood glucose concentration by means of a glucometer (Accu-Chek; Roche Diagnostics, Budaörs, Hungary), before and each 5 minutes in the first 80 minutes of the clamp process and each 10 minutes during the last 40-minute period. For fasting plasma insulin and CCK determination, blood samples were collected (in tubes of 1.0-mL volume containing 40 μL EDTA and 20 μL Trasylol; Bayer, Leverkusen, Germany) from the carotid artery immediately before commencement of the insulin infusion. The development of steady-state plasma hyperinsulinemia was confirmed by radioimmunoassay determinations from blood samples obtained between 80 and 90 minutes of the HEGC by 3 consecutive determinations divided by 3-minute intervals. Plasma CCK level was also measured during the HEGC steady state. The blood samples for both plasma insulin and CCK determinations were centrifuged (Centrifuge 5415R; Eppendorf, Hamburg, Germany) for 1 minute at 4°C and 10 000*g*, and the aliquots were frozen and stored at −70°C for succeeding determinations. The glucose infusion rate (GIR, in milligrams per kilogram per minute) needed to maintain the target blood glucose level during the steady state was referred to as a measure of HEGC insulin sensitivity.

2.5. Radioimmunoassay studies

Plasma insulin was determined by means of radioimmunoassay as described previously [12]. Plasma CCK

concentration was measured by a commercial CCK radioimmunoassay kit RK-069-04 (Phoenix Pharmaceuticals, Belmont, CA). The CCK antibody was raised against CCK octapeptide 26-33 (nonsulfated). The sensitivity of the assay was 1 pg per tube. The CD_{50} for the calibration curve was 35.44 pg per tube.

2.6. Study design

Sixteen animals were used throughout the experiments. The animals were randomly divided into 2 groups with 8 animals per group. One group was assigned to "systemic" whereas the other group was for "portal" insulin administration during HEGC. Each experiment commenced at 8:00 AM after an overnight fast. The animals were allowed a postsurgery stabilization period of 30 minutes. Blood samples were taken for plasma insulin, CCK, as well as blood glucose determination. Thereafter, the RIST method was performed to establish fasting whole-body insulin sensitivity (at low plasma insulin level). As the RIST method was completed and the fasting insulin sensitivity/resistance was determined, a continuous insulin infusion at a rate of 3 mU/(kg min) was started through one of the jugular or the spleen vein (systemic vs portal HEGC). The GIR was adjusted to maintain same blood glucose level as that maintained during the first RIST (Fig. 1). This hyperinsulinemic clamping was done to mimic postprandial hyperinsulinemia. When steady-state plasma insulin level had been reached and confirmed by 3 consecutive determinations (usually between 80 and 90 minutes into the HEGC procedure), the RIST was repeated (second RIST) and the insulin sensitivity was determined again. Plasma insulin and CCK level was measured at the following time points: (1) just before the first RIST (fasted state), (2) at the 13th minute of the first RIST (the time point of maximum GIR during the first RIST), (3) during the steady state of HEGC (simulation of the postprandial hyperinsulinemia), and (4) in the 13th minute of the RIST performed during the HEGC (Fig. 1).

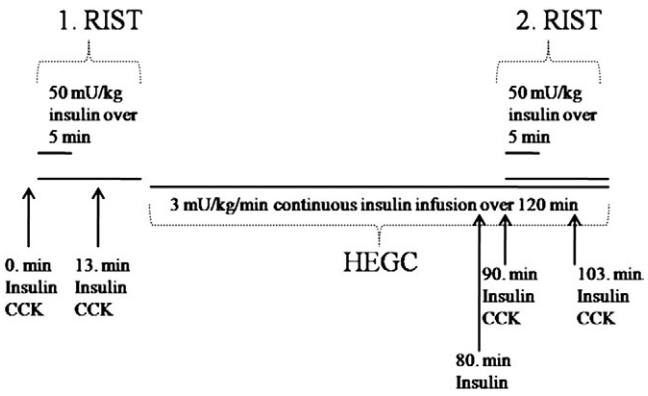

Fig. 1. Schematic diagram of the experimental design. In fasted animals, a RIST was performed followed by the HEGC. During the steady state of the HEGC, the RIST was repeated. Arrows indicate the time points when blood samples for insulin and CCK determination were taken.

Table 1
Changes in plasma insulin and CCK level during the experimental protocol

Plasma insulin	Fasted (μU/mL)	Systemic HEGC (μU/mL)	Portal HEGC (μU/mL)
Before RIST	11.9 ± 5.2	124.3 ± 17.2	131.8 ± 32.3
During RIST	87.8 ± 17.3	172.4 ± 19.7	170.6 ± 37.4
Plasma CCK	**Fasted (ng/mL)**	**Systemic HEGC (ng/mL)**	**Portal HEGC (ng/mL)**
Before RIST	2.2 ± 0.3	2.1 ± 0.2	1.9 ± 0.3
During RIST	1.9 ± 0.4	2.0 ± 0.4	2.1 ± 0.2

The bolus intravenous infusion of 50 mU/kg insulin evoked an elevation of plasma insulin level as expected. The insulin sensitivity determined by RIST method was as low as in the fasted state irrespective of the total systemic plasma insulin concentration. Neither systemic nor portal HEGC influenced plasma level of CCK. The data are means ± SD obtained from 8 animals per group.

2.7. Drugs and chemicals

Insulin (Humulin R) was purchased from Eli Lilly, thiopental sodium (Trapanal) was from BYK-Hungaris (Budapest, Hungary), and heparin (Heparibene Na 25000) was from ratiopharm (Ulm, Germany). All drugs were dissolved and diluted in isotonic saline solution.

2.8. Statistical analysis

The results are expressed as means ± SD obtained with 8 animals per group. The data were analyzed with repeated-measures analysis of variance followed by Student t test modified according to the Bonferroni method. Changes were considered statistically significant at P not exceeding .05.

3. Results

3.1. Changes in plasma insulin and CCK level throughout the experiment

The fasting plasma insulin and CCK levels were 11.9 ± 5.2 μU/mL and 2.2 ± 0.3 ng/mL, respectively (n = 16). The bolus infusion of 50 mU/kg insulin during the RIST caused elevation in the plasma insulin level to 87.8 ± 17.3 μU/mL. The continuous insulin infusion directly into the systemic circulation throughout the jugular vein during the HEGC caused elevation in the plasma insulin level to 124.3 ± 17.2 μU/mL (n = 8). The plasma insulin level moved up to 172.4 ± 19.7 μU/mL in response to the bolus insulin infusion during the second RIST (n = 8). When the continuous insulin infusion was administered throughout the spleen vein, the plasma insulin level increased to 131.8 ± 32.3 μU/mL (n = 8). In response to the second 50-mU/kg bolus intravenous insulin during the second RIST (n = 8), the plasma insulin level moved up to 170.6 ± 37.4 μU/mL.

Neither the 50 mU/kg bolus (during RIST with or without HEGC) nor the 3 mU/(kg min) continuous insulin (neither

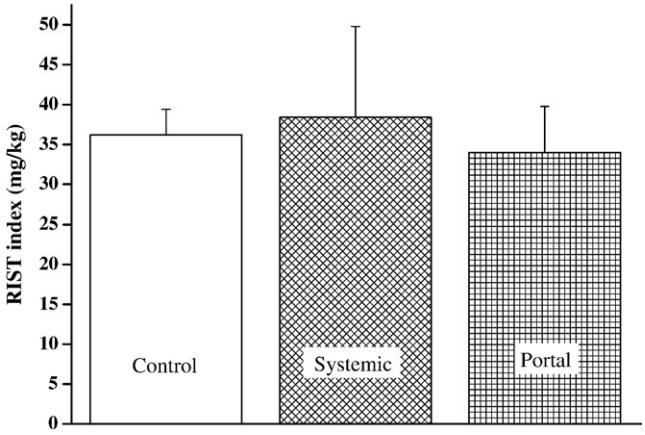

Fig. 2. Insulin sensitivity of the fasted animals (open column) and the insulin sensitivity during hyperinsulinemia caused by continuous systemic (hatched column) or portal (square column) insulin infusion at a constant rate of 3 mU mg/kg/min over 120 minutes. There are no significant differences among groups. The data are means ± SD obtained with 8 animals per group.

systemic nor portal) infusion modified the plasma CCK level (Table 1).

3.2. The effect of plasma insulin level on the insulin sensitivity as expressed by RIST index

In fasted animals (n = 16), the RIST index was 37.4 ± 3.1 mg/kg (Fig. 2). When the postprandial plasma insulin level was mimicked by means of 3-mU/(kg min) continuous insulin administration directly into the systemic circulation through the jugular vein, the RIST index performed during the steady-state hyperinsulinemia showed no changes as compared with the fasting state and was 39.7 ± 10.6 mg/kg (n = 8). When the same amount of insulin was infused throughout the portal circulation, the RIST index was as low as in the fasted state and was 35.7 ± 4.3 mg/kg (n = 8). The GIRs during the systemic and portal HEGC were 4.3 ± 1.1 and 3.8 ± 1.3 mg/(kg min), respectively.

4. Discussion

The present work was designed to explore one of the crucial steps of the HISS mechanism, the most potent endogenous insulin-sensitizing mechanism described to date, namely, whether hyperinsulinemia might serve as an initial step to promote a pathway that eventually leads to an increase in whole-body insulin sensitivity. Although we used the RIST method that is suitable to investigate the HISS mechanism as proposed by Lautt et al [13], we failed to confirm that hyperinsulinemia by itself was able to elicit insulin sensitization at least in fasted animals using RIST as an end point. Thus, the lack of evidence for insulin per se as a trigger for activation of insulin sensitization pathways is the major original finding of the study.

Our results are in accordance with those of previous studies revealing that the fasted state is characterized by low RIST values relative to those seen in fed animals, indicating insulin resistance (data shown in Fig. 2 and in Latour et al [2], Peitl and Szilvassy [7], Peitl et al [11], and Lautt et al [13]). Based on the previously suspected role of insulin in the HISS mechanism [1], it could have been expected that the artificial hyperinsulinemia evoked by continuous insulin infusion activated the HISS mechanism with an eventual improvement of whole-body insulin sensitivity. However, when euglycemic hyperinsulinemia was produced by HEGC to attain an increase in plasma insulin immunoreactivity resembling postprandial values (approximately 110 μU/mL) [14], no increase in insulin sensitivity was seen contrary to that suggested by Lautt's [8] hypothesis; that is, the whole-body insulin sensitivity remained as low as seen in the fasted animals with low plasma insulin levels. Nevertheless, eating a meal releases insulin from the pancreas that enters the portal circulation and subsequently the liver. Therefore, to mimic portal hyperinsulinemia, a feature of the postprandial state, insulin was infused into the spleen vein (portal HEGC) at the same rate as occurred during the systemic HEGC. Again, no change in insulin resistance was observed, despite the portal application of insulin.

It has long been recognized that an oral glucose load yielded a greater insulin secretory response than the same amount of glucose applied intravenously [15]. This at least in part was attributed to the release of CCK, a gastrointestinal peptide shown to play a pivotal role in activation of the postprandial insulin sensitization mechanism [7]. As reflected in the data in Table 1, hyperinsulinemia, no matter how induced, attained no change in plasma CCK level. Thus, hyperinsulinemia of the same magnitude as that seen after an ordinary meal in rats could not attain any significant increase in either plasma CCK level or whole-body insulin sensitivity.

Whatever the precise mechanism, the present results failed to confirm that hyperinsulinemia, either portal or systemic, serves as a primary trigger of any pathways leading to an increase in insulin sensitization in rats. Based on the findings presented, it is unlikely that postprandial hyperinsulinemia alone would be the basis of the HISS mechanism. What is more likely is that gastrointestinal peptides such as CCK form fundamental factors of endogenous insulin sensitization and release. Indeed, diseases such as celiac disease and sprue with known deficiency in CCK release are accompanied by diabetes and are often termed as *diabetic diarrhea* [16]. To explore the precise mechanism responsible for the HISS activation, including the investigation of the possible role of gastrointestinal regulatory peptides, more comprehensive research is required in the near future.

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