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# The prandial insulin sensitivity–modifying effect of vagal stimulation in rats

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**Abstract**

The effect of left cervical vagal nerve stimulation was studied on insulin sensitivity to test the proposed permissive insulin-sensitizing role of hepatic vagal parasympathetic efferent pathways in fasted and fed anesthetized rats. In fed animals, electrical stimulation (square impulses: 25 V, 5 Hz, 0.5 milliseconds over 15 minutes) of the vagal nerve induced hyperglycemia and an increase in plasma insulin immunoreactivity. Atropine (1.0 mg/kg intravenously) induced insulin resistance estimated by rapid insulin sensitivity testing. This was amplified when the vagal nerve was stimulated. The insulin-resistant state developed by fasting was not modified by either treatment with atropine or electrical stimulation. We conclude that both parasympathetic cholinergic and noncholinergic vagal efferents modulate postprandial neurogenic insulin sensitivity adjustments.


## 1. Introduction

A novel endogenous insulin-sensitizing mechanism proposed to be related to vagal parasympathetic efferent pathways was described by Lautt et al termed as the hepatic insulin–sensitizing substance (HISS) mechanism [1]. According to the original description of the HISS-related machinery, postprandial hyperinsulinemia activates some vagal-derived parasympathetic fibers in the anterior hepatic plexus that triggers the release of a currently undefined substance termed HISS, which enters the circulation and sensitizes peripheral tissues, predominantly skeletal muscle, to the hypoglycemic effect of insulin. This was essentially derived from the observation that either intraportal or systematic administration of atropine, a muscarinic receptor antagonist, decreased insulin sensitivity determined by the rapid insulin sensitivity test, a method developed by Lautt et al [2] in fed animals to a level seen in the fasting state [3]. Nevertheless, beyond atropine, inhibitors of cyclooxygenase, nitric oxide synthase, or adenylyl cyclase also attenuated the HISS-related postprandial increase in insulin sensitivity [4].

Our results obtained with hyperinsulinemic euglycemic clamping, the gold standard method for determination of whole body insulin sensitivity, did not show any effect of atropine or intraportal acetylcholine on the hypoglycemic effect of insulin in rats, whereas both hepatic denervation and inhibition of hepatic neural nitric oxide synthesis decreased insulin sensitivity similar to that produced by hepatic sensory denervation achieved by perineurial capsaicin desensitization of the anterior hepatic plexus. We therefore concluded that the HISS mechanism was of sensory nitrergic nature in both rats and rabbits with minimum involvement of parasympathetic efferent pathways at least when hyperinsulinemic euglycemic glucose clamp was used as end point [5,6]. To resolve a part of controversy between our results and those of Lautt's group, we studied if vagal efferent pathways sensitive to atropine modified either postprandial or fasting insulin sensitivity in anesthetized rats, using the rapid insulin sensitivity method proposed by Lautt et al, as the most appropriate one for studies on the HISS mechanism

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[7]. To activate the vagal nerve, we used direct electrical stimulation of the peripheral stump, the left cervical vagal nerve after acute nerve cut at parameters previously shown to activate the HISS mechanism [6]. These vagal fibers have been shown to comprise parasympathetic efferents running to the liver in the anterior hepatic plexus [8] known to underlie the HISS mechanism [1].

## 2. Materials and 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. The experimental protocol applied has been approved by the local ethical boards of the Universities of Debrecen, Hungary (DEMAB 007/2003).

### 2.2. Experimental animals and groups

The study was carried out with male Wistar rats weighing 250 to 350 g. They were housed in an animal room with 12-hour light and dark periods a day, temperature of 22°C to 25°C, humidity of 50% to 70% with 5 animals per pen. The animals were divided into 2 main experimental groups, that is, fed and fasted groups. The fed group was allowed to access to commercial laboratory chow and tap water ad libitum, whereas the fasted group was starved over a period of 16 hours preceding insulin sensitivity determination.

### 2.3. General operation procedure

The animals 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. Polyethylene catheters were introduced into the left jugular vein for insulin and glucose infusion and into the carotid artery for blood sampling and measurement of blood pressure by means of Statham P23 DB transducers attached to an Experimetria Ltd (Budapest, Hungary) TSZ06 electromanometer. The responses were recorded on a polygraph (Type RM, Beckman). To avoid blood coagulation, the animals were given intravenous (IV) heparin (100 IU/kg IV). Rectal temperature was kept at 37°C ± 0.5°C by a controlled infrared lamp (Experimetria Ltd). At the end of the experiments, the animals were killed by an overdose of thiopental sodium (100 mg/kg IV).

### 2.4. Electrical stimulation of the left cervical vagal nerve

As a part of the general operation procedure, the left cervical vagal nerve was prepared, cleaned of fat and adhering connective tissue, and subsequently cut. The peripheral stump was electrically stimulated with electrical square impulses (25 V, 5 Hz, 0.5 milliseconds) for 15 minutes. The electrical stimulation protocol and the insulin infusion were started simultaneously.

### 2.5. Rapid insulin sensitivity test

The rapid insulin sensitivity test (RIST) with 50 mIU/kg insulin was preformed as described by Lautt et al [2]. In brief, after a 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 (5.6 ± 0.1, 5.7 ± 0.1, and 5.5 ± 0.1 mmol/L). The total amount of glucose (expressed as milligram per kilogram of body weight) required to counteract the hypoglycemic effect of insulin infusion and to maintain the control blood glucose level produced the RIST index as an indicator of whole body insulin sensitivity.

### 2.6. Radioimmunoassay studies

Plasma insulin level was determined by means of radioimmunoassay using commercial insulin radioimmunoassay kit (RK 400 M, Institute of Isotopes, Budapest, Hungary). Both intra- and inter-assay variations were lower than 5%.

### 2.7. Experimental protocol

After a postsurgery 30-minute period of stabilization, blood samples (<0.5 mL) were taken from the carotid artery for plasma insulin and glucose measurements in both main experimental groups. Subgroups were then formed to study the effect of atropine/its vehicle with 6 animals in each. Further groups of 6 animals were used to study the effect of vagal nerve stimulation on insulin sensitivity in the presence or absence of atropine. For assessment of the effect of vagal stimulation on plasma insulin immunoreactivity, samples were taken before and in the last minute of the stimulation procedure.

### 2.8. Drugs and chemicals

Atropine was purchased from EGIS (Budapest, Hungary), and thiopental sodium from BYK-Hungaria kft (Budapest, Hungary). Thiopental sodium was dissolved and diluted in isotonic saline.

### 2.9. Statistical analysis

The results are expressed as means ± SEM obtained with 6 animals per group. The data were analyzed with repeated-measures analysis of variance followed by Student $t$ test modified according to Bonferroni method. Changes were considered statistically significant at $P \leq .05$.

## 3. Results

### 3.1. Effect of atropine and electrical stimulation of the vagal nerve on insulin sensitivity

In fed animals, atropine significantly reduced the RIST index by about 40% from the control of 178.8 ± 12.3 to 106.3 ± 12.8 mg/kg body weight. In fasted animals, the

control RIST index was 42.4 ± 4.2 mg/kg and atropine did not produce any further change (47.5 ± 8.5 mg/kg). Vagal nerve stimulation induced an increase in blood glucose level, which remained high in spite the exogenous insulin bolus used for the RIST procedure in fed animals; thus, the RIST index could not be determined in this group. In fed animals with atropine treatment, vagal stimulation dramatically (ie, with a strong significance) decreased insulin sensitivity compared to those without vagal stimulation (Fig. 1). In fasted animals, the low RIST index did not change in response to either atropine or electrical stimulation of the vagal nerve (Fig. 1). The mean arterial blood pressure did not change in either fed or fasted animals during the RIST procedure and/or vagal nerve stimulation (110 ± 12 and 108 ± 13 mm Hg) as compared with the pre-RIST/vagal stimulation values (114 ± 16 mm Hg). Similarly, either procedure was without effect on heart rate (313 ± 23 and 289 ± 32 1/min vs control: 308 ± 33 1/min).

### *3.2. Effect of electrical stimulation of the left cervical trunk of the vagal nerve on blood glucose level and plasma insulin immunoreactivity*

When the fed animals were subjected to electrical stimulation of the left cervical vagal nerve (25 V, 5 Hz, 0.5 milliseconds, 15 minutes), it caused an immediate increase in blood glucose level, which remained stable over the entire stimulation period and returned to baseline values within 15 minutes after cessation of stimulation (Fig. 2). The plasma insulin level was 106.1 ± 8.1 μIU/mL before and 137.8 ± 7.1 μIU/mL ($P \leq .05$) immediately after electrical stimulation of the vagal nerve (Fig. 2). After

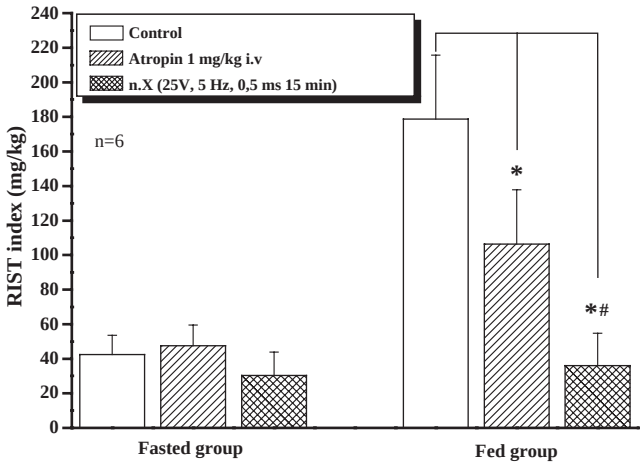


Fig. 1. Effects of atropine (1 mg/kg IV) on the hypoglycemic action of insulin as expressed by means of RIST index in the fed and fasted groups of rats. Electrical stimulation of the left cervical vagal nerve (25 V, 5 Hz, 0.5 milliseconds, 15 minutes) did not change the insulin sensitivity in fasted animals but further decreased in fed rats. The results are means ± SE obtained with 6 animals in each group. Asterisk indicates a significant difference between corresponding values and controls; number sign, a significant difference between atropine-treated and vagal stimulation groups.

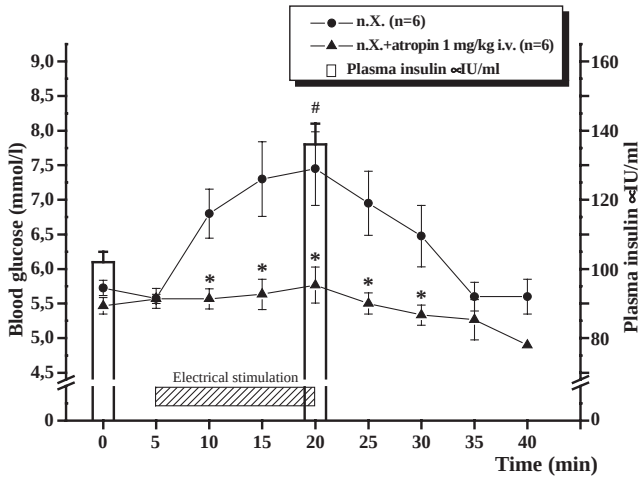


Fig. 2. The effect of electrical stimulation of the left cervical vagal nerve (25 V, 5 Hz, 0.5 milliseconds, 15 minutes) on the blood glucose and on the plasma insulin immunoreactivity levels in solvent- and atropine-pretreated fed rats. The results are means ± SE obtained with 6 animals in each group. Asterisk indicates a significant difference between corresponding values and controls.

atropine administration (1 mg/kg IV), electrical stimulation of the left vagal nerve caused a decrease in blood glucose level and required to start glucose infusion to maintain the control blood glucose level (Fig. 2) even in the absence of exogenous insulin. In fasted animals, neither blood glucose level (3.6 ± 0.8 mmol/L) nor plasma insulin (16.5 ± 5.6 μIU/mL) changed significantly after vagal stimulation.

In fasted and fed animals, the pre-RIST plasma insulin immunoreactivity values were 16.5 ± 5.6 and 106 ± 8.1 μIU/mL, whereas those measured immediately after cessation of the 50 mIU/kg insulin bolus were 74.1 ± 10.4 and 173.1 ± 14.1 μIU/mL, respectively.

## 4. Discussion

The present work was designed to find direct evidence for the postprandial whole body insulin-sensitizing effect of parasympathetic vagal efferent pathways, first proposed by Lautt's group, based on results from pharmacological studies [4]. Therefore, we used the same species (rat) and the same insulin sensitivity end point (the RIST index) as suggested by Lautt [7] himself as an optimum method to study the HISS paradigm. However, we used direct stimulation of the vagus nerve at parameters previously shown to attain activation of the HISS mechanism [6].

The results show that electrical stimulation of the left cervical vagal nerve, which gives parasympathetic fibers of the anterior hepatic plexus [8], known to underlie the HISS mechanism [1], yields an increase in blood glucose level with an increase in plasma insulin immunoreactivity in fed anesthetized rats. This response is substantially modified by atropine, that is, hypoglycemia evolves with no change in plasma insulin level when vagal stimulation is performed

after 1.0 mg/kg atropine applied IV. As far as the effect of vagal stimulation on postprandial whole body insulin sensitivity is concerned, because an immediate hyperglycemic response was seen after commencement of nerve stimulation, the RIST method could not be performed in this experimental paradigm. Considering that the nerve stimulation–induced hyperglycemic response was accompanied by an increase in plasma insulin level, the overall effect of vagal stimulation on insulin sensitivity at the stimulation parameters applied could virtually be referred to as an insulin-desensitizing one. Nevertheless, assuming that the nerve stimulation–induced abrupt increase in blood glucose level is possibly attributed to an increase in hepatic glucose production that in turn stimulates insulin release, the hyperglycemic response to vagal stimulation may occur independent of any change in insulin sensitivity. Atropine converted the hyperinsulinemic hyperglycemic effect of vagal stimulation to a hypoglycemic response with no change in plasma insulin immunoreactivity, which can be referred to as an atropine-induced insulin sensitization at least in the presence of electrical vagal stimulation. This latter effect would have been interpreted as a noncholinergic insulin-sensitizing one if the RIST index had not shown decreased whole body insulin sensitivity (Fig. 1).

Our results seem to confirm those of Lautt's group, in terms of the difference in RIST index obtained in fed and fasted animals [4]. Nevertheless, as shown from the results in Fig. 2, plasma insulin immunoreactivity in fed animals is much higher than in those from the fasted group; therefore, it makes very difficult the interpretation of data obtained with the RIST index as a measure of tissue insulin sensitivity. The RIST method was originally developed to replace the insulin tolerance test with a convenience of avoiding hypoglycemic events [9]. Essentially, this method is referred to as a frequently sampled euglycemic clamp after an insulin bolus producing very high plasma insulin levels, with a disadvantage of provoking several central nervous system–related and hemodynamic adjustments [10-13]. Moreover, this method is far less widely accepted than the hyperinsulinemic euglycemic glucose clamp method, the gold standard procedure to determine whole body insulin sensitivity in both clinical and experimental settings. Considering that taking a meal produces hyperinsulinemia by itself, and that the RIST method produces the measure of insulin sensitivity by means of the amount of glucose to be infused to compensate for the hypoglycemic effect of insulin, irrespective of whether the effect of endogenous or exogenous insulin is being compensated, the reliability of the RIST method as an insulin sensitivity determination procedure, at least without information on plasma insulin levels, is questionable. Nevertheless, in our studies, the ratio of fed over fasted plasma insulin immunoreactivity ratio was 2.33, whereas the fed/fasted RIST ratio was 4.23, which might reflect an additional, really insulin-sensitizing mechanism than that would have resulted from a single difference in plasma insulin levels.

To the best of our knowledge this work is the first designed to study the effect of direct vagal stimulation on whole body insulin sensitivity reflected in the RIST index. Because the stimulation procedure produced such a dramatic increase in blood glucose level in spite the 50 mIU/kg exogenous insulin bolus, the RIST procedure could not be executed. This suggests that the stimulation either released glucose from endogenous sources (possibly from hepatic glycogen) and/or produced a dramatic decrease in tissue insulin sensitivity. Based on the current data available, it is not possible to provide a satisfactory explanation on either. Previous studies agreed in that intraportal acetylcholine resulted in an increase or no change in tissue insulin sensitivity determined by means of either hyperinsulinemic euglycemic glucose clamping [6,14] or the RIST method [15]. The controversy may at least in part be explained by that most vagal fibers belong to sensory afferents with several neurotransmitters, predominantly peptides in rats. Some of these peptides are known to either decrease (eg, calcitonin gene-related peptide) [16] or increase (eg, somatostatin) [17] insulin sensitivity. Nevertheless, when vagal stimulation was performed in animals pretreated with atropine, the RIST procedure was possible to perform although it revealed RIST values as low as those seen in the fasted group. Thus, it is suggested that cholinergic pathways do not seem to be major contributors to neurogenic insulin sensitization in rats.

Whatever the precise mechanism, the results failed to strengthen the hypothesis that vagal cholinergic parasympathetic efferent pathways significantly contribute to postprandial endogenous insulin sensitization even using the RIST method as an end point.

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