Abstract

Aims: Hemodynamic nitrate tolerance has been shown to result in an insulin resistant state. We studied whether nitrate tolerance induced by a 7-day continuous exposure to transdermal nitroglycerin influenced the meal-induced insulin sensitization phenomenon in rabbits.

Methods: Changes in insulin sensitivity in response to feeding in conscious rabbits were determined by rapid insulin sensitivity test, in both nitrate tolerant and non-tolerant animals. In a separate series of experiments with anaesthetized rabbits with or without nitrate tolerance, the hyperinsulinaemic euglycaemic glucose clamping methods was used to study the effect of intraportal infusion of cholecystokinin (CCK) on whole body insulin sensitivity.

Results: Rabbits with normal feeding exhibited a 46 ± 6 % increase in insulin sensitivity as compared to their matching fasting control. A 7-day period of treatment with patches releasing 0.07 mg nitroglycerin per hour yielded nitrate tolerance and a state of insulin resistance and no increase in insulin sensitivity in response to food. Intraportal infusion of CCK8 (0.3-3.0 μg/kg over 20 min) resulted in a dose-dependent increase in insulin sensitivity in normal but not in nitrate tolerant fasted anaesthetized animals.

Conclusions: Nitrate tolerance blocks both the meal-induced insulin sensitization phenomenon and the insulin sensitizing effect of intraportal cholecystokinin.

VASCULAR

Block by nitrate tolerance of meal-induced insulin sensitization in conscious rabbits.

Á. Bajza, L. Drimba, J. Németh PhD, G. Oroszi PhD, R. Pórszász PhD, B. Peitl PhD, Z. Szilvássy PhD DSc

Dept. Pharmacology and Pharmacotherapy, University of Debrecen, H-4032 Debrecen, 98. Nagyerdei blvd.

Running title: nitrate tolerance and insulin resistance

Corresponding author: Barna Peitl MD, PhD;

98. Nagyerdei blvd.

Debrecen

Hungary H-4032

Tel/Fax: +36 52 427 899

e-mail: barna.peitl@king.pharmacol.dote.hu

Abstract

Aims: Hemodynamic nitrate tolerance has been shown to result in an insulin resistant state. We studied whether nitrate tolerance induced by a 7-day continuous exposure to transdermal nitroglycerin influenced the meal-induced insulin sensitization phenomenon in rabbits.

Methods: Changes in insulin sensitivity in response to feeding in conscious rabbits were determined by rapid insulin sensitivity test, in both nitrate tolerant and non-tolerant animals. In a separate series of experiments with anaesthetized rabbits with or without nitrate tolerance, the hyperinsulinaemic euglycaemic glucose clamping methods was used to study the effect of intraportal infusion of cholecystokinin (CCK) on whole body insulin sensitivity.

Results: Rabbits with normal feeding exhibited a 46 ± 6 % increase in insulin sensitivity as compared to their matching fasting control. A 7-day period of treatment with patches releasing 0.07 mg nitroglycerin per hour yielded nitrate tolerance and a state of insulin resistance and no increase in insulin sensitivity in response to food. Intraportal infusion of CCK8 (0.3-3.0 μg/kg over 20 min) resulted in a dose-dependent increase in insulin sensitivity in normal but not in nitrate tolerant fasted anaesthetized animals.

Conclusions: Nitrate tolerance blocks both the meal-induced insulin sensitization phenomenon and the insulin sensitizing effect of intraportal cholecystokinin.

Keywords: nitrate tolerance, insulin resistance, type 2 diabetes, rabbit, hyperinsulinaemic euglycaemic glucose clamp, rapid insulin sensitivity test, cholecystokinin

Conflicts of interest: none

Introduction

Organic nitrates constitute the mainstay therapy of angina pectoris and congestive heart failure. In addition to the therapeutically exploited cardiovascular effects, nitroglycerin, the prototype of nitrates used in human therapy has been shown to attain an insulin sensitizing effect in both experimental animals[1] and healthy volunteers[2]. For convenience of chronic nitrate therapy, transdermal nitroglycerin delivery systems were developed to provide sustained effects. The resulting continuously high plasma concentration, however, was found to induce hemodynamic tolerance with an attenuation of the antianginal effect[3,4]. Subsequently, it has also been shown that hemodynamic nitrate tolerance blocks several endogenous adaptive mechanisms underpinned by nitric oxide (NO) release such as the anti-ischemic effect of myocardial preconditioning[5,6], the non-adrenergic, non-cholinergic relaxation of gastrointestinal sphincters[7] and yields a state of insulin resistance[1]. The latter was suspected to somehow relate to the meal-induced insulin sensitization phenomenon first described by Sadri & Lautt[8], since it was widely accepted that the phenomenon was the most effective NO-dependent endogenous insulin sensitizing mechanism.

In a recent study, it has been described that the meal-induced endogenous insulin sensitizing mechanism can be blocked by proglumide, a cholecystokinin (CCK) receptor antagonist[9]. Results from another study with rats revealed that functional integrity of CCK1 receptors is a prerequisite for the development meal-induced insulin sensitization[10]. Since NO of neural origin has been shown to be involved in the effects of CCK on gastrointestinal motility and secretion[11,12], the present work was concerned with the possibility that the CCK-NO pathway was vulnerable to a state of hemodynamic nitrate tolerance. Thus, we devoted a series of experiments with rabbits to study whether nitrate tolerance, a state shown to deteriorate responses to both endogenous and exogenous NO, influenced the insulin sensitizing effect of either an exposure to meal or CCK infused into the portal vein.

Materials & Methods

Ethical Issues and General Procedures

The investigation conforms to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and to the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985). The experimental protocol applied has been approved by the local ethical board of the University of Debrecen, Hungary (license number: 6/2007/DE MÁB)

Experimental animals

Sixty-eight, adult, male New Zealand white rabbits, weighing 3 – 3.2 kg (Charles – Rivers Laboratories, Isaszeg, Hungary), housed in an animal room (12 – 12hour light/dark periods a day, temperature of 22 – 25°C, humidity of 50 – 70%) with one animal per pen (Techniplast, Italy), fed commercial laboratory chow (Charles-River Laboratories, Isaszeg, Hungary) and tap water ad libitum, were used throughout.

Study Design

After a 7-day period of acclimatization, the rabbits were tested for hypotensive response to a previously established intravenous 30 µg/kg test dose of nitroglycerin (Pohl-Boskamp GmbH, Hohenlockstedt, Germany) applied over a period of 1 min[13]. The maximum decrease in blood pressure produced the data for evaluation. The rabbits exhibiting no significant decrease in blood pressure to the nitroglycerin test dose were excluded from further studies. The animals were then randomized into three main experimental groups. The group 1 animals entered the rapid insulin sensitivity test (RIST) series of experiments to study the effect of meal-induced insulin sensitization in starved animals (group 1a) and in those

with a re-exposure to food (group 1b). In this series of studies, changes in whole body insulin sensitivity were assessed by the RIST method in conscious animals, an approach specifically proposed to investigate the effect of food on insulin sensitivity[14]. The second main group of conscious animals entered the hyperinsulinaemic euglycaemic glucose clamp (HEGC) studies. In this latter series of experiments, whole body insulin sensitivity was determined in rabbits either starved (group 2a) or re-exposed to food (group 2b) by means of hyperinsulinaemic euglycaemic glucose clamping, a gold standard method for insulin sensitivity determinations in both human and whole animals[15]. The series of experiments with the third group of animals was devoted to study the effect of intraportal CCK infusion on insulin sensitivity in anaesthetized rabbits (thiopental 25 mg/kg i.v.) after a 24-h period of fasting. In these animals, CCK octapeptide sulphate (CCK8) was infused into the portal vein during the steady state of HEGC at 0.3, 1.0 and 3.0 μg/kg/h over 20 min in a cumulative manner. During the last minute of the highest infusion rate of CCK, hepatic samples were taken for cGMP determinations. For control to this series of rabbits served those with (4 rabbits) and without nitrate tolerance (4 rabbits), which received vehicle infusion not succeeded by CCK8. This series of experiments was to confirm that intraportal CCK8 infusion could attain an insulin sensitizing effect in fasting rabbits (Fig. 1).

Each main group was further randomized in the same way as to whether the animals received either transdermal nitroglycerin (NG) patches releasing approximately 0.07 mg/kg/h nitroglycerin (Nitroderm TTS 5, Novartis Hungaria Budapest, Hungary) or matching placebo patches continuously over 7 days. Each patch was replaced daily with a new one. Either the RIST procedure (group 1 animals) or HEGC (group 2) was commenced 1 hour after removal of the last patch. After completion of either the RIST or HEGC determinations, we confirmed the presence or absence of nitrate tolerance by measuring changes in mean arterial blood pressure in response to the intravenous test dose (30 μg/kg body weight) of nitroglycerin in

the same way as accomplished prior to randomization to the main experimental groups. This occurred 4 hours after removal of the last (7th) patch.

Verification of nitrate tolerance

Animals were given transdermal nitroglycerin or placebo patches over 7 days to induce vascular tolerance to glyceryl trinitrate (GTN)[16]. Development of vascular tolerance to GTN was confirmed on the 7th day by testing endothelium free carotid artery rings for isometric tension as described previously[5,6]. Rings of 5 mm in length were precontracted with an EC_{50} concentration of norepinephrine in addition to a resting tension of 10 mN. The rings were then exposed to cumulative concentrations of GTN in half-log increments. The GTN concentrations required to produce half-maximal relaxation were 0.091 ± 0.012 mM in vehicle treated, non-tolerant rings versus 1.72 ± 0.19 mM in GTN-treated, tolerant ones ($P<0.05$).

Rapid Insulin Sensitivity Test (RIST)

Rapid insulin sensitivity test as previously described by Lautt et al.[17] was preformed after minor modification according to different experimental species. In brief, an arterial and a venous cannula (Vygonüle V G22, Vygon GmbH &Co., Aachen, Germany) were introduced into the arteria and vena auricularis, respectively. Thereafter, animals were left for 30 minutes in order to stabilize their physiological parameters. Then arterial blood samples were taken at every 5 minutes for blood glucose determination. The mean blood glucose level of three consecutive measurements was referred to as the control value; afterwards, infusion of 50 mU/kg insulin over 5 minutes was started. In order to counteract the hypoglycaemic effect of insulin, glucose infusion was also commenced and its rate was adjusted to maintain the control blood glucose level. The total amount of glucose (expressed as mg/kg body weight)

required to counteract the hypoglycaemic effect insulin and to maintain the control blood glucose level was expressed as RIST index, the indicator of whole body insulin sensitivity[9,18].

Hyperinsulinaemic Euglycaemic Glucose Clamp (HEGC)

On one side, the arteria and vena auricularis was cannulated. The glucose and the insulin infusion lines were connected to a venous cannula by a 3-way stop cock (Tro-venoflow 3, Troge Medical GmbH, Hamburg, Germany). After a 30 min stabilization period, human regular insulin (NOVO Nordisk, Copenhagen, Denmark) was infused (Syringe Pump 11 Plus – Harvard Apparatus, Holliston, Massachusetts, USA) at a constant rate of 10 mU kg-1 via one of the venous infusion line over 120 min. This insulin infusion rate yielded plasma insulin immunoreactivity of 100 ± 5 μU/ml during the steady state. Blood samples (0.2 ml) were taken from the arterial cannula for blood glucose determination at 10 min intervals. Blood glucose concentration was maintained constant (5.5 ± 0.5 mmol/L) by a variable rate of glucose infusion (Optima VS – Fresenius Kabi AG, Bad Homburg, Germany) via a second venous infusion line. When blood glucose had stabilized for at least 20 min, we defined this condition as steady state. This occurred within 100 min succeeding commencement of the insulin infusion. In the steady state, additional blood samples (0.2 ml) were taken for plasma insulin determination three times at 10-min intervals. The glucose infusion rate (mg/kg/min) during steady state was used to characterize insulin sensitivity[15]. Each clamp determination was done after a preceding 12-hour period of fasting. The arterial cannula besides serving for blood sampling also was used for monitoring the arterial blood pressure and heart rate. For this latter purpose, the arterial cannula was connected to a Statham P23DB transducer attached to an electromanometer (Experimetria Kft. Budapest, Hungary), as described previously [19].

Measurement of hepatic cGMP level

Liver cGMP levels were determined from hepatic samples taken immediately after cessation of CCK8 infusion by means of radioimmunoassay as described previously[19] using Amesham kits. The inter- and intra-assay variations did not exceed 5%. Sampling was done from animals with and without nitrate tolerance.

Drugs and Chemicals

The CCK octapeptide was purchased from Sigma-Aldrich Ltd. (Budapest, Hungary). Nitroglycerin patches were from Novartis (Basle, Switzerland) and nitroglycerin for intravenous use was from the Pohl-Boskamp GmbH (Hohenlockstedt, Germany). Glucose infusion was from Teva (Debrecen, Hungary). Human regular insulin was purchased from Novo Nordisk (Copenhagen, Denmark).

Statistical analysis

Data are expressed as means ± S.D. of the mean were analysed with ANOVA followed by a modified t-test for paired data. P values were adjusted according to Bonferroni's method[20].

Results

Hemodynamic nitrate tolerance achieved by transdermal NG

As shown by data in Table 1, transdermal NG produced a significant decrease in mean arterial blood pressure after the 1st patch on with no change in heart rate. The 3rd and the 7th patch on did not produce any change in these parameters. Thirty min after removal of the last patch, there were no changes in blood pressure and heart rate.

In untreated animals, the intravenous test dose of NG (30 µg/kg) decreased mean arterial blood pressure from 83 ± 5.1 and 86 ± 4.9 to 61 ± 4.4 and 60 ± 5.3 mmHg in those recruited into the groups being treated with NG releasing patches and placebo patches, respectively ($P<0.01$ for each). In rabbits treated with placebo patches over 7 days, the intravenous NG test dose decreased the ’patch off’’ blood pressure from 83 ± 4.8 to 60 ± 3.6 mmHg ($P<0.01$), whereas in animals treated with active patches, no change in blood pressure was seen in response to the test NG dose. This confirmed the fact of development of hemodynamic nitrate tolerance in the group of rabbits treated with active patches.

Methaemoglobin formation did not exceed 1% over the study in either tolerant or non-tolerant animals.

Insulin sensitivity reflected in the RIST index in fasted and re-fed animals with and without nitrate tolerance

It is seen from data in Fig 2. that the RIST index was almost doubled in animals with placebo patches re-exposed food as compared to that measured with their fasting littermates. In rabbits with established hemodynamic nitrate tolerance, however, re-exposure to food was without effect on the RIST index. Fasting RIST values were lower in nitrate tolerant animals than those in the non-tolerant ones (Fig. 2).

Insulin sensitivity measured by HEGC in fasted and re-fed animals with and without nitrate tolerance

The data in Fig. 2. show that the glucose infusion rate needed to maintain euglycaemia at clamped hyperinsulinaemia increased with re-exposure to food as compared to corresponding fasting values in non-tolerant animals. This indicated an increase in whole

body insulin sensitivity in response to food. However, the food-induced insulin sensitization phenomenon was completely abolished in animals made nitrate tolerant.

The insulin sensitizing effect of CCK8 during HEGC

As shown by data in Fig. 3., CCK8 increased the rate of glucose infusion necessary to maintain euglycaemia during the steady state of the HEGC procedure in a dose-dependent manner. In rabbits with nitrate tolerance the CCK8 infusion produced no change in insulin sensitivity.

The effect of CCK infusion on hepatic cGMP in rabbits with nitrate tolerance

Hepatic cGMP level increased from 0.22 ± 0.07 and 0.13 ± 0.04 to 1.11 ± 0.09 and 0.19 ± 0.04 pmol/mg wet tissue weight in response to 3 µg/kg/h CCK8 infusion ($P<0.05$ for both vs vehicle) in non-tolerant and tolerant animals, respectively.

Discussion

The results show that the state of hemodynamic nitrate tolerance achieved by a 7-day continuous exposure to transdermal nitroglycerin is associated with the loss of the meal-induced insulin sensitization phenomenon in conscious rabbits. Beyond this original observation, the result confirm our previous results in that whole body insulin sensitivity determined in fasting rabbits is also significantly impaired when the animals are made tolerant to the hypotensive effect of transdermal nitroglycerin. An additional original observation is that the insulin sensitizing effect of intraportal CCK8 is impaired in nitrate tolerance. Considering that CCK can be considered a physiological trigger of post-prandial insulin sensitization, the present results represent a step forward in understanding mechanisms underlying the effect of meal on whole body insulin sensitivity in intact animals.

The concept that a deficiency in the NO-cGMP pathway of whatever origin yields a state of insulin resistance is not new. Petrie et al.[21] measured insulin-mediated glucose disposal in healthy volunteers with the hyperinsulinaemic, euglycaemic clamp technique and defined the relationship between whole body sensitivity and an estimate of NO production. The major conclusion drawn was that a positive correlation existed between endothelial NO synthesis and insulin sensitivity in healthy volunteers. Subsequently, Piatti et al.[22] reported that non-diabetic individuals with a positive family history of type 2 diabetes were insulin resistant, with deficient cGMP production at higher plasma NO concentrations, than that seen in healthy volunteers without a family history of diabetes. Under experimental conditions, allowing precise pharmacological analysis, NO synthase inhibitors apparently induced insulin resistance, and intraportal administration of NO donors restored physiological insulin sensitivity in rats[8,23], rabbits[24] and guinea-pigs[25]. In addition, studies with conscious rabbits revealed that the development of nitrate tolerance, a state known to be characterized by a substantial decrease in cGMP formation yielded insulin resistance[1]. This latter is strongly supported by results of the present work in terms of detecting relative insulin resistance in nitrate tolerant animals both in the fasting state and after meal.

We think that the straightforward original observation of the present work is that the meal-induced insulin sensitizing mechanism is seriously impaired in nitrate tolerant rabbits. It is suspected that the meal-induced insulin sensitizing phenomenon is underlain by the release of a chemically undefined substance from the liver in response to food that sensitizes peripheral tissues, predominantly the skeletal muscle to the hypoglycaemic effect of insulin[26]. The substance has therefore been termed as a hepatic insulin sensitizing substance (HISS). Regarding the experimental approaches to study the HISS mechanism, Lautt et al. proposed the use of the rapid insulin sensitivity test[27] instead of hyperinsulinaemic euglycaemic glucose clamping, the gold standard method of whole body insulin sensitivity determinations

in the clinical practice[15]. The RIST method utilizes hyperinsulinaemia produced by a short-term insulin infusion (usually 5 min), the hypoglycaemic effect of which is compensated by a succeeding longer lasting glucose infusion[17]. During the hyperinsulinaemic clamp method, however, plasma insulin immunoreactivity is maintained at a level 10 or more times higher than corresponding fasting values with confirmation by succeeding radioimmunoassay determinations and accompanied by continuous glucose infusion. Assuming that the HISS mechanism was suspected to be activated at least in part by hyperinsulinaemia[28], it might be postulated that a much higher degree of the HISS mechanism activation is achieved by clamping (because of the long-lasting stable hyperinsulinaemia) than by the RIST method. Nevertheless, in a recent study, we could not confirm the concept of hyperinsulinaemia-induced activation of the HISS pathway[29]. Whatever the method used, the results clearly demonstrate that whole body insulin sensitivity is significantly increased when the animals are re-exposed to food after a preceding period of fasting and that this food-induced insulin sensitization phenomenon is blocked in nitrate tolerance.

The present findings also seem to indirectly support the results by Peitl et al.[10] in that CCK is of major influence on the postprandial insulin sensitization pathway. In their study, proglumide, a CCK receptor antagonist blocked the phenomenon of meal-induced insulin sensitization, and no change in insulin sensitivity was seen in response to food intake in animals without functioning CCK-1 receptors. As reads from data in Fig. 3 of the present work, stepwise increments in intraportal infusion of CCK8 produce a dose-dependent increase in whole body insulin sensitivity, an effect also completely blocked by nitrate tolerance. This is another original observation of the present work. Moreover, the results of experiments using HEGC and RIST together as end-points suggest for the first time that not only the post-prandial, but baseline insulin sensitivity is decreased by nitrate tolerance. The precise

mechanism, however, responsible for the vulnerability of CCK effects to either NO synthase inhibition[11] or nitrate tolerance, cannot be depicted based on results currently available.

In a previous work carried out with isolated rabbit sphincter of Oddi preparations, we found that the contractile effect of CCK was not modified by nitrate tolerance [7]. Therefore, a simple interaction between CCK receptor function and nitrate tolerance is unlikely to explain changes in CCK effects in other experiments. Under *in vivo* conditions, however, the complex motor effect of CCK strongly involves in the release/effect of NO [30], thus, the cross tolerance between exogenous and endogenous NO was striking at experimental paradigm different from the present design. Otherwise, the role of NO in hepatic insulin sensitization has been confirmed several times by various authors in various species[8,24,31]. Of course, it is more than possible that oxidative stress plays a role in this cross tolerance, possibly by neutralizing NO of whatever origin, by various biochemical mechanisms, and considering that reactive oxygen species are important mediators of nitrate tolerance, separate experiments were not directed towards analysing the role of oxidative stress on food-induced insulin sensitization in nitrate tolerance. In rabbits, changes in the metabolism of cGMP seem to be another important factor in desensitization of NO-dependent processes in nitrate tolerance. The cGMP phosphodiesterase inhibitors such as cicletanine and zaprinast were able to reverse haemodynamic nitrate tolerance in isolated vessels and gastrointestinal smooth muscle (unpublished observations). Based on the current results, it is impossible to provide explanation whether nitrate tolerance by what mechanism deteriorates the insulin sensitizing effect of intraportal CCK8. As CCK could achieve a much lower increase in hepatic tissue cGMP levels in tolerant than in non-tolerant animals, an increase in the breakdown of cGMP in nitrate tolerance cannot be excluded.

Conclusions

Taken together, our results demonstrate for the first time, that the development of hemodynamic nitrate tolerance deteriorates the meal-induced insulin sensitization phenomenon in addition to decreasing whole body insulin sensitivity in fasting conscious and anaesthetized rabbits. Regarding the mechanisms behind, nitrate tolerance seems to seriously impair the insulin sensitizing effect of CCK. Considering that CCK is a major regulator of appetite[32], post-prandial glucose production[33] and gastrointestinal motility and secretion[30], the interaction between nitrate tolerance and the effects of CCK released may explain metabolic complications of nitrate therapy[34].

Acknowledgements

This study was supported by the Hungarian National Office for Research and Technology (GOP-1.1.2-07/1-2008-0004; TÁMOP-4.2.2.-08/1-2008-0014, OM-00174/2008 and GOP-1.1.1-07/1-2008-0032 and GOP-1.2.1-08-2009-0023) and the Hungarian Scientific Research Fund (OTKA-75965).

References

1. Bajza A, Peitl B, Nemeth J, Porszasz R, Rabloczky G, Literati-Nagy P, Szilvassy J, and Szilvassy Z. Development of insulin resistance by nitrate tolerance in conscious rabbits. J Cardiovasc Pharmacol 2004 Mar;43:471-476.

2. Kovacs P, Szilvassy Z, Hegyi P, Nemeth J, Ferdinandy P, and Tosaki A. Effect of transdermal nitroglycerin on glucose-stimulated insulin release in healthy male volunteers. Eur J Clin Invest 2000 Jan;30:41-44.

3. Parker JO and Fung HL. Transdermal nitroglycerin in angina pectoris. Am J Cardiol 1984 Sep;54:471-476.

4. Luke R, Sharpe N, and Coxon R. Transdermal nitroglycerin in angina pectoris: efficacy of intermittent application. J Am Coll Cardiol 1987 Sep;10:642-646.

5. Szilvassy Z, Ferdinandy P, Bor P, Jakab I, Szilvassy J, Nagy I, Lonovics J, and Koltai M. Loss of preconditioning in rabbits with vascular tolerance to nitroglycerin. Br J Pharmacol 1994 Aug;112:999-1001.

6. Ferdinandy P, Szilvassy Z, Csont T, Csonka C, Nagy E, Koltai M, and Dux L. Nitroglycerin-induced direct protection of the ischaemic myocardium in isolated working hearts of rats with vascular tolerance to nitroglycerin. Br J Pharmacol 1995 Aug;115:1129-1131.

7. Sari R, Szilvassy Z, Jakab I, Nagy I, and Lonovics J. Cross tolerance between nitroglycerin and neural relaxation of the rabbit sphincter of Oddi. Pharmacol Res 1998 Jun;37:505-512.

8. Sadri P and Lautt WW. Blockade of hepatic nitric oxide synthase causes insulin resistance. Am J Physiol 1999 Jul;277:G101-G108.

9. Peitl B and Szilvassy Z. The inhibitory effect of proglumide on meal-induced insulin sensitization in rats. Metabolism 2007 Jul;56:863-864.

10. Peitl B, Dobronte R, Drimba L, Sari R, Varga A, Nemeth J, Pazmany T, and Szilvassy Z. Involvement of cholecystokinin in baseline and post-prandial whole body insulin sensitivity in rats. Eur J Pharmacol 2010 Oct;644:251-256.

11. Sari R, Peitl B, Kovacs P, Lonovics J, Palvolgyi A, Hegyi P, Nagy I, Nemeth J, Szilvassy Z, and Porszasz R. Cyclic GMP-mediated activation of a glibenclamide-sensitive mechanism in the rabbit sphincter of Oddi. Dig Dis Sci 2004 Mar;49:514-520.

12. Palvolgyi A, Sari R, Nemeth J, Szabolcs A, Nagy I, Hegyi P, Lonovics J, and Szilvassy Z. Interplay between nitric oxide and VIP in CCK-8-induced phasic contractile activity in the rabbit sphincter of Oddi. World J Gastroenterol 2005 Jun;11:3264-3266.

13. Szilvassy Z, Ferdinandy P, Nagy I, Jakab I, and Koltai M. The effect of continuous versus intermittent treatment with transdermal nitroglycerin on pacing-induced preconditioning in conscious rabbits. Br J Pharmacol 1997 Jun;121:491-496.

14. Lautt WW. Postprandial insulin resistance as an early predictor of cardiovascular risk. Ther Clin Risk Manag 2007 Oct;3:761-770.

15. DeFronzo RA, Tobin JD, and Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979 Sep;237:E214-E223.

16. Silver PJ, O'Connor B, Cumiskey WR, van AG, Hamel LT, Bentley RG, and Pagani ED. Inhibition of low Km cGMP phosphodiesterases and Ca+(+)-regulated protein kinases and relationship to vasorelaxation by cicletanine. J Pharmacol Exp Ther 1991 Apr;257:382-391.

17. Lautt WW, Wang X, Sadri P, Legare DJ, and Macedo MP. Rapid insulin sensitivity test (RIST). Can J Physiol Pharmacol 1998 Dec;76:1080-1086.

18. Peitl B, Dobronte R, Nemeth J, Mezey G, Kovacs P, Paragh G, and Szilvassy Z. The prandial insulin sensitivity-modifying effect of vagal stimulation in rats. Metabolism 2005 May;54:579-583.

19. Szilvassy Z, Ferdinandy P, Bor P, Jakab I, Lonovics J, and Koltai M. Ventricular overdrive pacing-induced anti-ischemic effect: a conscious rabbit model of preconditioning. Am J Physiol 1994 May;266:H2033-H2041.

20. Wallenstein S, Zucker CL, and Fleiss JL. Some statistical methods useful in circulation research. Circ Res 1980 Jul;47:1-9.

21. Petrie JR, Ueda S, Webb DJ, Elliott HL, and Connell JM. Endothelial nitric oxide production and insulin sensitivity. A physiological link with implications for pathogenesis of cardiovascular disease. Circulation 1996 Apr;93:1331-1333.

22. Piatti PM, Monti LD, Zavaroni I, Valsecchi G, Van PC, Costa S, Conti M, Sandoli EP, Solerte B, Pozza G, Pontiroli AE, and Reaven G. Alterations in nitric oxide/cyclic-GMP pathway in nondiabetic siblings of patients with type 2 diabetes. J Clin Endocrinol Metab 2000 Jul;85:2416-2420.

23. Porszasz R, Legvari G, Pataki T, Szilvassy J, Nemeth J, Kovacs P, Paragh G, Szolcsanyi J, and Szilvassy Z. Hepatic insulin sensitizing substance: a novel 'sensocrine' mechanism to increase insulin sensitivity in anaesthetized rats. Br J Pharmacol 2003 Jul;139:1171-1179.

24. Porszasz R, Legvari G, Nemeth J, Literati PN, Szolcsanyi J, and Szilvassy Z. The sensory nitrergic nature of the hepatic insulin sensitizing substance mechanism in conscious rabbits. Eur J Pharmacol 2002 May;443:211-212.

25. Szilvassy J, Sziklai I, Sari R, Nemeth J, Peitl B, Porszasz R, Lonovics J, and Szilvassy Z. Neurogenic insulin resistance in guinea-pigs with cisplatin-induced neuropathy. Eur J Pharmacol 2006 Feb;531:217-225.

26. Lautt WW. The HISS story overview: a novel hepatic neurohumoral regulation of peripheral insulin sensitivity in health and diabetes. Can J Physiol Pharmacol 1999 Aug;77:553-562.

27. Reid MA, Latour MG, Legare DJ, Rong N, and Lautt WW. Comparison of the rapid insulin sensitivity test (RIST), the insulin tolerance test (ITT), and the hyperinsulinemic euglycemic clamp (HIEC) to measure insulin action in rats. Can J Physiol Pharmacol 2002 Aug;80:811-818.

28. Lautt WW. Practice and principles of pharmacodynamic determination of HISS-dependent and HISS-independent insulin action: Methods to quantitate mechanisms of insulin resistance. Med Res Rev 2003 Jan;23:1-14.

29. Peitl B, Dobronte R, Nemeth J, Pankucsi C, Sari R, Varga A, and Szilvassy Z. Meal-induced enhancement in insulin sensitivity is not triggered by hyperinsulinemia in rats. Metabolism 2009 Mar;58:328-332.

30. Grider JR. Role of cholecystokinin in the regulation of gastrointestinal motility. J Nutr 1994 Aug;124:1334S-1339S.

31. Moore MC, Satake S, Baranowski B, Hsieh PS, Neal DW, and Cherrington AD. Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs. Am J Physiol Endocrinol Metab 2002 Feb;282:E286-E296.

32. Cummings DE and Overduin J. Gastrointestinal regulation of food intake. J Clin Invest 2007 Jan;117:13-23.

33. Cheung GW, Kokorovic A, Lam CK, Chari M, and Lam TK. Intestinal cholecystokinin controls glucose production through a neuronal network. Cell Metab 2009 Aug;10:99-109.

34. Fayers KE, Cummings MH, Shaw KM, and Laight DW. Nitrate tolerance and the links with endothelial dysfunction and oxidative stress. Br J Clin Pharmacol 2003 Dec;56:620-628.

Figure legends

Figure 1 shows the study design. Abbreviations: RIST: Rapid Insulin Sensitivity Test; HEGC: Hyperinsulinaemic Euglycaemic Glucose Clamping; the term "starved animals" is referred to as a 16-h period of fasting before either the RIST or the HEGC procedure; the re-fed rabbits were fasted over 14 h, than they were re-allowed to access to food for 2 h preceding the insulin sensitivity determination had been carried out; "+" indicates rabbits treated with nitroglycerin patches, "-" indicates rabbits treated with placebo patches.

Figure 2 shows the effect of nitrate tolerance on meal-induced insulin sensitization in rabbits. Whole body insulin sensitivity was determined in rabbits either starved (open bars or fasted) or re-exposed to standard food (squares columns or re-fed) by means of both hyperinsulinaemic euglycaemic glucose clamping (HEGC), and the rapid insulin sensitivity test (RIST). The term "Tolerant" means that the animals were made tolerant to the hypotensive effect of an intravenous dose of nitroglycerin (30 µg/kg) by a preceding continuous 7-day exposure to transdermal patches releasing approximately 0.07 mg/kg/h nitroglycerin. The "Non-tolerant" animals were exposed to matching placebo patches over 7 days and exhibited a significant decrease in blood pressure in response to the intravenous 30 µg/kg nitroglycerin test dose. Each column represents data obtained with a subgroup of 6 rabbits. The data are means ± S.D. The asterisk: Re-fed vs fasted at $P \leq 0.05$; +: Tolerant vs non-tolerant at $P \leq 0.05$.

Figure 3 shows the effect of nitrate tolerance on the insulin sensitizing effect of intraportal cholecystokinin octapeptide (CCK) in anaesthetized rabbits. CCK was infused into the portal vein in stepwise increments at doses 0.3-3.0 mg/kg/h during steady state of HEGC. The term

“Tolerant” means that the animals were made tolerant to the hypotensive effect of an intravenous dose of nitroglycerin (30 µg/kg) by a preceding continuous 7-day exposure to transdermal patches releasing approximately 0.07 mg/kg/h nitroglycerin. The “Non-tolerant” animals were exposed to matching placebo patches over 7 days and exhibited a significant decrease in blood pressure in response to the intravenous 30 µg/kg nitroglycerin test dose. The data are means ± S.D. obtained with subgroups of 6 rabbits The asterisk denotes a significant difference between GIR (glucose infusion rate) values obtained after a CCK dose vs vehicle at $P \leq 0.05$; + tolerant vs non tolerant at $P \leq 0.05$.

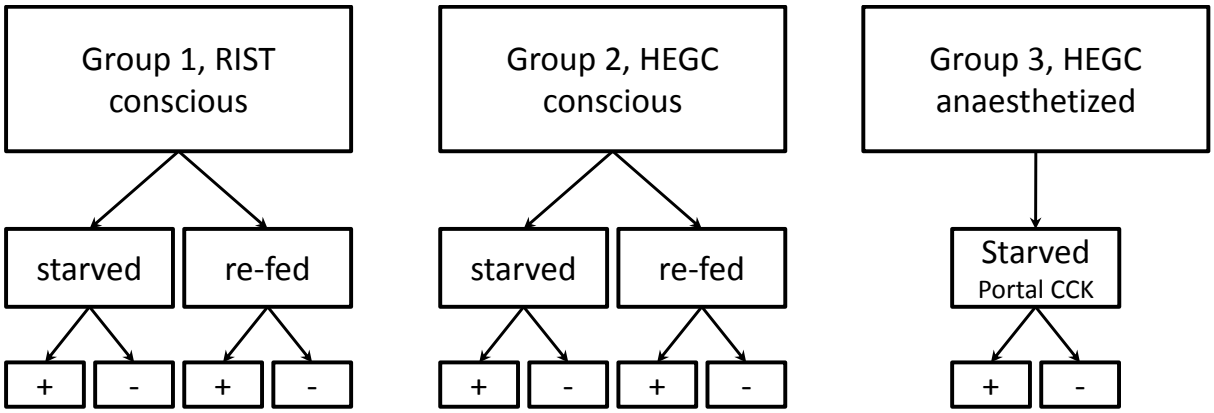

Figure 1.

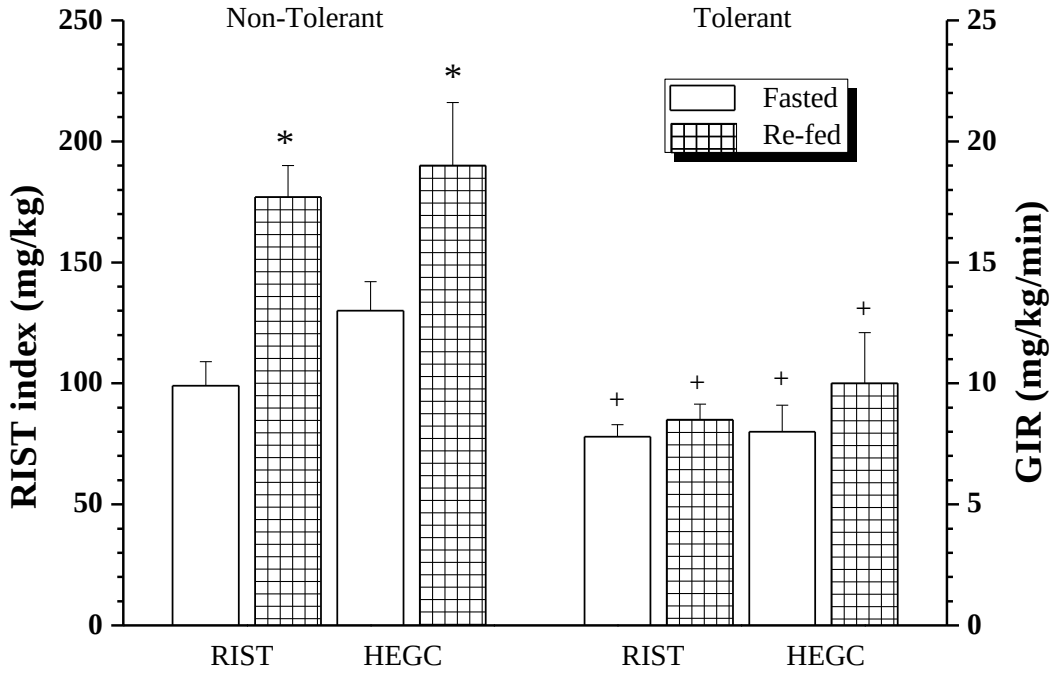

Figure 2.

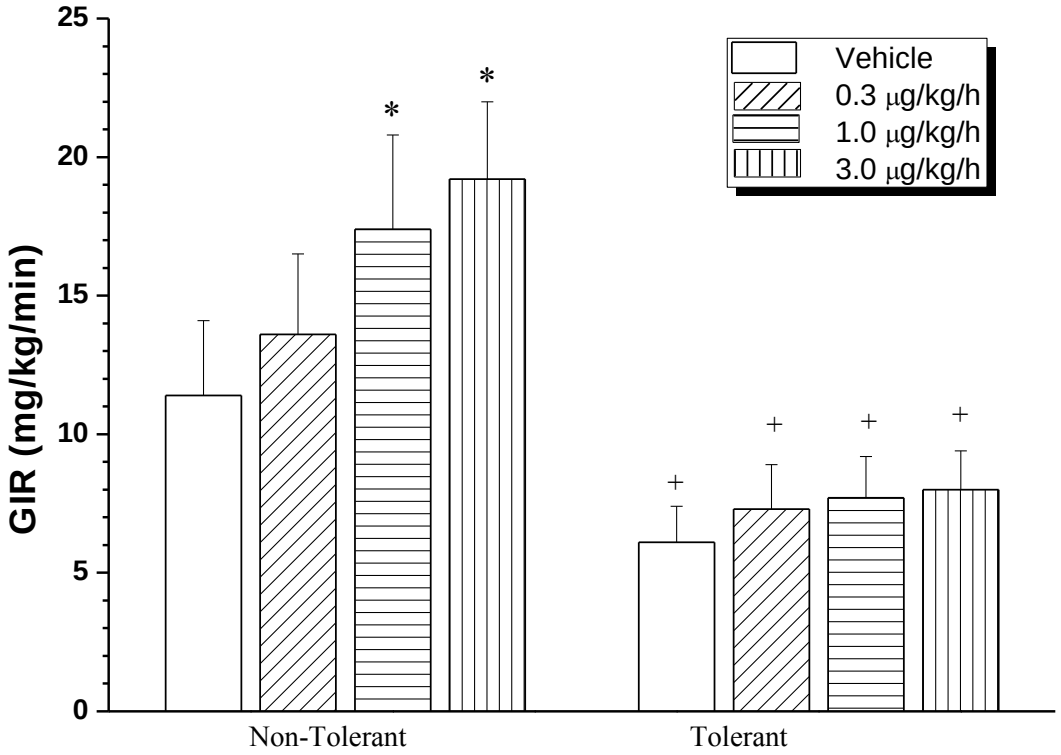
25
20
15
10
5
0
GIR (mg/kg/min)
Vehicle
0.3 μg/kg/h
1.0 μg/kg/h
3.0 μg/kg/h
*
*
+
+
+
+
Non-Tolerant
Tolerant

Figure 3.

Table

Mean Arterial Blood Pressure (mm Hg)					
Groups	**Before patch**	**1st 'patch on**	**3rd 'patch on'**	**7th 'patch on'**	**4 h after 'patch off '**
NG Patch	83 ± 5.1	69 ± 3.2*	85 ± 4.3	84±5.5	85±6.0
Placebo	86 ± 4.9	85 ± 4.4	84 ± 5.5	82 ± 5.4	83 ± 4.8
Heart Rate (b.p.m.)					
Groups	**Before Patch**	**1st 'patch on'**	**3rd 'patch on'**	**7th 'patch on'**	**3 h after 'patch off'**
NG Patch	236 ± 16	242 ± 18	244 ± 19	277 ± 14*	273 ± 14*
Placebo	241 ± 22	248 ± 16	237 ± 22	248 ± 20	235 ± 18

Table 1. shows the effect of Transdermal Nitroglycerin (NG) on Arterial Blood Pressure and Heart Rate in Conscious Rabbits The data are means ± SD obtained with 6 animals per group. *Significantly different from "before" values at $P < 0.05$. The data indicate maximum changes in blood pressure and heart rate that occurred within 30 min after patch on. Patch off values were determined 4 h after removal of the last patch.