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Investigation of the role of nitric oxide in the development of insulin resistance

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1. Introduction

Studies associated with diabetes are nowadays one of the most dynamically developing field of the medical sciences. Diabetes is a so called „plague” of our era because the number of diabetic patients in the world rises rapidly, and it is the main risk factor of the cardiovascular diseases. Therefore it has a great importance in the public health. WHO reported that there are 345 million people affected in the world. It is one of the civilization diseases in the developed countries and it has basic influence on the duration and the quality of our life.

The diabetes and its complications as the ischaemic heart disease, the cerebrovascular diseases, the renal failure, the polineuropathy, the diabetic eyes and the retinopathy are all at the top of the list of the disease and the death list respectively of the modern society. Originally the diabetes was known as a uniform disease. The latter was shown that diabetes is heterogenic represents a „group” of diseases with a background of insufficiant effect of insulin. Patophysiologically the disease can be classified into two groups. The first type (the „earlier” name: insulin dependent diabetes mellitus, IDDM), when the primary cause is the reduced production of insulin beacause of the fault of the pancreatic beta cells, and the second type (non insulin dependent diabetes mellitus, NIDDM), where the periferal tissues, mainly the striated muscular tissue and the adipose tissue are insensible against the effect of insulin.

Insulin resistance by definition is a state when the normal amount of insulin produces subnormal effect.

It is important to note that insulin resistance without diabetes is the starting point of the troubles of the cardiovascular and it represents an independent risk factor of coronary diseases.

Measurement of insulin

The main three targets of insulin are the adipose tissue, the muscular tissue and the liver, consequently the insulin resistance developes in these tissues or organs.

Central insulin resistance

The glycogenolysis is the production of glucose in liver from glycogen and is reduced by insulin. This effect doesn’t occurs or it is not efficient under pathological conditions, when the

glucose production of the liver is increased, and if even elevated level of insulin doesn't decrease the glucose production, the insulin resistance of the liver cells is developed.

Periferal insulin resistance

The main glucose consumer tissue is the skeletal muscle. In insulin resistance the decreased periferal glucose consumption and the increased glucose production of the liver lead to hyperglycaemia.

Methods of measurements of the effect of peripheral insulin

Insulin resistance can be measured by different methods. The glucose level decreasing effect of insulin is realized in two points of attack, by the stimulation of peripheral glucose consumption or with the inhibition of glucose production (downtake) of the liver. Based on these facts we can say that in insulin resistance the decreased peripheral glucose consumption and/or the increased glucose production of liver lead to the hyperglycaemia. There are a lot of methods to measure the effect of insulin. Two groups of these methods are:

- Indirect methods:
    a. Oral glucose tolerance test (OGTT)
    b. Intravenous glucose tolerance test (IVGTT)
- Direct methods
    a. Intravenous insulin tolerance test (IVITT)
    b. Insulin supression test
    c. Insulin glucose clamp (EGC)

The nitrates

The organic nitrate compounds, since more than a century are widely used drugs of cardiovascular diseases. They are most frequently used in the therapy of angina pectoris and the congestive heart failure. In addition, in the last years the nitroglycerine and its derivatives

play an increasingly important role in the medical treatments of several non cardiovascular diseases as well, for example in the disorders of gastrointestinal motility, in glaucoma or in the potential disorders. Moreover, it has been shown to attain an insulin senzitizing effect in both experimental animal and healthy adult volunteers. Nevertheless, the effect of nitrate therapy is unic and problematic, during its application we have to consider numerous obstacles, mainly the nitrate tolerance and the nitroglycerine rebound, because of their practical importance.

For convenience of the prolonged use of nitrate therapy the transdermal nitroglycerin delivery system has been developed, which produces long-lasting and constant effect. Nevertheless, it results continuously high plasma concentration, that causes hemodynamic nitrate tolerance, while the anti-anginal effect is decreasing.

It becomes obvious that hemodynamic nitrate tolerance blocks several endogenous adaptive mechanisms such as the antiischemic effect of myocardial preconditioning, the non-adrenerg, non-cholinerg relaxation of gastrointestinal sphincters and yields a state of insulin resistance. It was supposed that hemodynamic nitrate tolerance is somehow related to increased meal-induced insulin sensitivity. Later this hypothesis was widely accepted as the most effective NO-dependent endogenous insulin sensitizing mechanism.

The cholecystokinin

Cholecystokinin (CCK) is a peptide belonging to a gastrine family of gastro-intestinal system. It’s secretion comes from the duodenum and from the so called I-cells of the proximal part of jejunum. In addition CCK is also present in the other nervous structures of the organisms, such as around the Langerhans-islands of pancreas, where probably plays a role in the regulation of mechanisms of β- and α-cells, because increases the release of insuline and glucagon.

CCK develops its effect in the target cells by bounding to the corresponding CCK-receptors (CCK-1 and CCK-2). There are some specific CCK-receptors in the pancreas, gall bladder, oesophageal sphincter, ileum and in the colon, in addition in the brain and in some of the peripheral nerves.

As it is known, the application of antagonists allows us to study substances that play roles in the regulation of certain physiological functions. The antagonists block the bounding of test substance to its receptor, while doesn't provoke any biological effect itself, resulting decresased or totally inhibited effect. The most important CCK-receptor antagonists are derivatives of antibiotics (asperlicine), derivatives of benzodiazepine (devazepide) and the derivatives of aminoacides (proglumide).

2. Aims

Nitric oxide has emerged as an important regulatory molecule with diverse functions in the cardiovascular system, in imunological processes and also in the nervous system. In the blood vessels NO mediates the endothelium-dependent vasodilatation, and in the central and peripheral nervous system NO acts as a so-called „unusual neurotransmitter". It has been shown that the systemic inhibition of NO-synthase causes insulin resistance, which can be counteract by intraportal administration of 3-morpholinosydnonimine (SIN-1) – a nitric oxide donor. This suggests that at an appropriate dosing schedule, NO donors might serve as drugs that increase insulin sensitivity.

Transdermal nitroglycerine at a dose used for angina prophylaxis suppresses glucose-stimulated insulin release with an increase in insulin sensitivity. This observation seems to support the concept of nitrergic regulation of peripheral insulin sensitivity, with the possibility of therapeutic use of nitrates in the management of insulin resistance and/or either type of diabetes. The prolonged use of nitrates, however, is often associated with the development of tolerance with an attenuation of the therapeutic effect. Moreover, endogenous nitrergic mechanisms have also been shown to seriously impaired in the state of nitrate tolerance.

The meal-induced endogenous insulin sensitizing mechanism can be blocked by proglumide, a cholecystokinin (CCK) receptor antagonist. Moreover, revealed that functional integrity of CCK 1 receptors is a prerequisite for the development of meal-induced insulin sensitization, since in rats with geneticaly insufficient CCK 1 receptor not only the post-prandial, but also the baseline insulin sensitivity is decreased. It has been shown that NO of neural origin is

involved in the effects of CCK on gastrointestinal motility and secretion, and the CCK-NO pathway is vulnerable to a state of nitrate tolerance.

Setting out from these considerations our aims were the followings:
1. comparison of effects of acute and continuous 7-day nitroglycerin treatments and the study of the development of insulin resistance in conscious rabbits,
2. study of the effect of nitrate tolerance and the potencial role of CCK in meal-induced insulin sensitization phenomenon in rabbits.

3. Materials and methods

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).

3.1. Comparison of the effects of acute and subchronic (7 days) nitroglycerin treatment and the study of developement of insulin resistance in conscious rabbits

Adult male New Zealand white rabbits (Charles-River Laboratories, Isaszeg, Hungary), weighing 3 to 3.2 kg, housed in animal room (12-hour light/dark periods a day, temperature of 22 to 25 ºC, humindity of 50 to 70%) with 1 animal per pen (Techniplast, Italy), fed commercial laboratory chow (Charles-River Laboratories, Isaszeg, Hungary) and tap water ad libitum, were used throughout. The animals underwent surgery after a 2-week adaptation period.

Surgery
Surgery was performed under aseptic conditions. The rabbits were anaesthetized with an intravenous bolus of 10 mg/kg diapzepam (Sigma, St Louis, MO) and 5 mg/kg ketamine (EGIS Pharmaceuticals Ltd., Budapest, Hungary). Lidocaine (EGIS, Hungary) was given subcutaneously for local pain relief as described previously. Polyethylene catheters were

inserted into two major branches of the jugular vein for insulin and glucose infusion and to obtain venous blood samples (maximum volume 0.5 ml) for methemoglobin determinations by means of multiwavelength photometric analysis using an ABL 625 System (Radiometer A/S, Copenhagen NV, Denlarg). A polyethylene tube connected to a Statham P23 DB transducer (Gould, Balainvilliers, France) and an EXPERIMETRIA (EXP-2, Budapest, Hungary) multiscriptor was inserted into the left carotid artery to measure mean arterial blood pressure. The catheters were exteriorized through the back of the neck as described. A small catheter also was inserted into the central artery of the right ear for arterial blood sampling. These lines were kept patent by filling them with sodium heparin solution (100 IU/ml).

Intravenous Glucose Disposal

The rabbits were given an intravenous bolus of 0.5 g/kg glucose dissolved in isotonic sodium chloride in 15 ml volume over 2 minutes by means of an infusion pump (Braun, Melsungen, Germany). Arterial blood samples were collected before infusion as well as at 10, 30, 60, 120, and 180 minutes after infusion for blood glucose and plasma insulin determination. Glucose was measured by the glucose oxidase method; plasma insulin immunoreactivity was determined by means of radioimmunoassay (RIA) using IZINTA (Isotope Institute, Budapest, Hungary) insulin RIA kits as described.

Hyperinsulinemic Euglycemic Glucose Clamp Studies (HEGC)

Human regular insulin was infused at a constant rate (13 mU/kg, NOVO Nordisk, Copenhagen, Denmark) via one of the venous catheters over 120 minutes. This insulin infusion yielded plasma insulin immunoreactivity of 100 ± 5 μU/ml in the steady state (see below). Blood samples (0.3 ml) were taken from the arterial cannula for blood glucose concentration at 10-minute intervals. Blood glucose concentration was maintained constant (5.5 ± 0.5 mmol/l) by a variable rate of glucose infusion via the second venous cannula. When blood glucose had stabilized for at least 30 minutes, we defined this condition as steady state. In the steady state, additional blood samples (0.5 ml) were taken for plasma insulin determination at 10-minute intervals. The glucose infusion rate (mg/kg/min) during steady state was used to characterize insulin sensitivity.

Induction of Hemodynamic Nitrate Tolerance

Hemodynamic nitrate tolerance was induced by continuous exposure to transdermal nitroglycerin as described. In brief, exposure of the rabbits to transdermal patches releasing

approximately 0.07 mg/kg/h nitroglycerin (Nitroderm TTS 5, Ciba Hungaria, Budapest, Hungary) over 7 days results in the lack of the hypotensive response to an intravenous test dose (30 μg/kg) of nitroglycerin, which produces a more than 20 % decrease in mean arterial blood pressure in control animals. The hypotensive response, however, is preserved after a 6-hour treatment with transdermal nitroglycerin. Thus, in the present work, a 6-hour treatment with transdermal nitroglycerin was used to study the effect of nitroglycerin in the *nontolerant* state, whereas a continuous 7-day exposure to TTS 5 patches was used to study the effect of nitrate tolerance on the variables investigated.

Study Design

One week after surgery, the rabbits were randomized to three major experimental groups. Group 1 (n = 12) animals entered the glucose disposal study, whereas Group 2 (n = 24) and Group 3 (n = 24) rabbits were used for hyperinsulinemic euglycemic clamp experiments. Group 1 rabbits were further randomized as to whether they received either transdermal patches releasing approximately 0.07 mg/kg/h nitroglycerin (Nitroderm TTS 5, Ciba Hungaria, Budapest, Hungary) or matching placebo patches continuously over 7 days. (Each patch was replaced daily with a new one.) Six hours after removal of the last patch, the intravenous glucose disposal study was commenced.

Group 2 animals were randomized in the same way. In this group of rabbits, the hyperinsulinemic euglycemic glucose clamp studies were started 6 hours after removal of the last patch.

Group 3 rabbits were divided into four subgroups; six animals received TTS 5 patches over 6 hours in the third hour of which the hyperinsulinemic euglycemic clamp study was commenced (ie, in the presence of transdermal nitroglycerin in *non-tolerant* state). The preceding 3-hour exposure to transdermal nitroglycerin was necessary to overcome problems resulting from an initial decrease in blood pressure due to “patch on” that could have influenced insulin sensitivity through a secondary baroreflex activation. Another subgroup of six animals received transdermal nitroglycerin over 7 days and hyperinsulinemic clamp studies occurred on the last day (ie, in the presence of transdermal nitroglycerin in the *tolerant* state). The control group received matching placebo patches over the corresponding periods. After completion of the glucose disposal or the insulin clamp studies, we confirmed the presence or absence of nitrate tolerance by measuring changes in blood pressure in response to the intravenous test dose of nitroglycerin (30 μg/kg) as described previously.

Statistical analysis

Data are expressed as means ± standard deviation (SD) of the mean and were analyzed with ANOVA followed by a modified *t* test for paired data. *P* values were adjusted according to Bonferroni method.

3.2. Study of the effect of nitrate tolerance and the potencial role of CCK of meal-induced insulin sensitization in conscious rabbits

Study design

After a 7-day period of acclimatization, the rabbits weretested for hypotensive response to a previously established intravenous 30 mg/kg test dose of NG (Pohl-Boskamp GmbH, Hohenlockstedt, Germany) applied over a period of 1 minute. The maximum decrease in blood pressure produced the data for evaluation. The rabbits exhibiting no significant decrease in blood pressure to the NG test dose were excluded from further studies. The animals were then randomized into 3 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 reexposure 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.

The second main group of conscious animals entered the hyperinsulinemic euglycemic glucose clamp (HEGC) studies. In this latter series of experiments, whole-body insulin sensitivity was determined in rabbits either starved (group 2a) or reexposed to food (group 2b) by means of HEGC, a gold standard method for insulin sensitivity determinations in both human and in vivo animal studies.

The series of experiments with the third group of animals was devoted to study the effect of intraportal CCK infusion on insulin sensitivity in anesthetized rabbits (25 mg/kg of thiopental intravenously) after a 24-hour period of fasting. In these animals, CCK octapeptide sulfate (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 minutes in a cumulative manner. During the last minute of the highest infusion rate of CCK, hepatic samples were taken for cyclic guanosine monophosphate (cGMP) determinations. The control animals, both the nitrate tolerant (4 animals) and nontolerant rabbits (4 animals), were infused with vehicle solution instead of CCK8. This

series of experiments was to confirm that intraportal CCK8 infusion could attain an insulin-sensitizing effect in fasting rabbits.

Each main group was further randomized in the same way as to whether the animals received either transdermal NG patches releasing approximately 0.07 mg/kg/h NG (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 NG in the same way as accomplished before randomization to the main experimental groups. This occurred 4 hours after the removal of the last (seventh) patch.

Verification of Nitrate Tolerance

Animals were given transdermal NG or placebo patches over 7 days to induce vascular tolerance to glyceryl trinitrate (GTN). Development of vascular tolerance to GTN was confirmed on the seventh day by testing endothelium-free carotid artery rings for isometric tension as described previously. Rings of 5 mm length were precontracted with an half maximal effective concentration (EC_{50}) 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 nontolerant rings versus 1.72 ± 0.19 mM in GTN-treated tolerant ones ($P < 0.05$).

Rapid Insulin Sensitivity Test (RIST)

RIST 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 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 3 consecutive measurements was referred to as the control value; afterward, infusion of 50 mU/kg of insulin over 5 minutes was started. To counteract the hypoglycemic 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 milligrams per kilogram of body weight) required to counteract the hypoglycemic effect of insulin and to maintain the control blood glucose level was expressed as RIST index, the indicator of whole-body insulin sensitivity.

Hyperinsulinemic Euglycemic Glucose Clamp (HEGC)

On one side, the arteria and vena auricularis were cannulated. The glucose and the insulin infusion lines were connected to a venous cannula by a 3-way stop cock (Trovenoflow 3; Troge Medical GmbH, Hamburg, Germany). After a 30-minute stabilization period, human regular insulin (Novo Nordisk, Copenhagen, Denmark) was infused (Syringe Pump 11 Plus; Harvard Apparatus, Holliston, MA) at a constant rate of 10 mU/kg via one of the venous infusion line over 120 minutes. 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-minute 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 minutes, we defined this condition as steady state. This occurred within 100 minutes succeeding commencement of the insulin infusion. In the steady state, additional blood samples (0.2 ml) were taken for plasma insulin determination 3 times at 10-minute intervals. The glucose infusion rate (mg/kg/min) during the steady state was used to characterize insulin sensitivity. 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.

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 using Amersham kits. The interassay and intraassay variations did not exceed 5 %. Sampling was done from animals with and without nitrate tolerance.

Statistical Analysis

Data are expressed as means ± SDs and were analyzed by analysis of variance followed by a modified *t* test for paired data. *P* values were adjusted according to the Bonferroni method.

4. Results

4.1. Comparison of the effects of acute and subchronic (7 days) nitroglycerin treatment and the study of developement of insulin resistance in conscious rabbits

Effect of Hemodynamic Nitrate Tolerance on Baseline Hemodynamics and Methemoglobin Formation

Transdermal nitroglycerin produced a transient decrease in mean arterial blood pressure in the first 30 minutes after the patch had been applied with no change in heart rate. A 7-day continuous exposure to transdermal nitroglycerin, however, produced an increase in heart rate irrespective of the presence or absence of the patches. The increase in heart rate was statistically significant from the third day of continuous patch on with 277 ± 11, 280 ± 14, 275 ± 9, and 281 ± 15 b.p.m. on the third, fourth, fifth, and sixth days, respectively ($P < 0.05$ versus control for each). Baseline mean arterial blood pressure did not change over the 7-day period irrespective of the presence or absence of active or placebo patches. Methemoglobin formation was below 1 % over the whole experimental period and did not change with the development of hemodynamic nitrate tolerance (data not shown).

Effect of Nitrate Tolerance on Glucose Disposal

It is seen that an intravenous glucose load (0.5 mg/kg) increased blood glucose level to a similar degree in *tolerant* and control animals. However, both pre-load or post-load plasma insulin levels were significantly higher in *tolerant* than in control rabbits at each time point

Effect of Nitrate Tolerance on Insulin Sensitivity

The glucose infusion rate to maintain euglycemia (5.5 mmol/l) at clamped hyperinsulinemia (100 μU/ml) was significantly lower in *tolerant* than in control (placebo-treated) rabbits.

Effect of Transdermal Nitroglycerin on Insulin Sensitivity in the Presence or Absence of Nitrate Tolerance

Acute treatment with transdermal nitroglycerin significantly increased insulin sensitivity reflected in an increase in glucose infusion rate to maintain euglycemia in animals with active "patch on" as compared with that in rabbits with placebo patches. However, a 7-day exposure to active patches producing hemodynamic nitrate tolerance produced resistance to the hypoglycemic effect of insulin irrespective of the presence or the absence of nitroglycerin patches on the seventh day.

The results confirm our previous finding that a 7-day continuous exposure to transdermal patches releasing approximately 0.07 mg/kg/h nitroglycerin produces hemodynamic nitrate tolerance in conscious rabbits. The 'tolerant' state is characterized by the lack of hypotensive response to an intravenous bolus of nitroglycerin, which is known to produce a marked decrease in blood pressure in 'non-tolerant' rabbits. The results also show that nitrate tolerance is not confined to an attenuation of the vasodilatory effect of nitroglycerin but also disturbs the regulation of carbohydrate metabolism by decreasing insulin sensitivity. This is suggested by results from experiments at two different paradigms:

1. plasma insulin immunoreactivity increased substantially in the 'tolerant' animals during glucose disposal and
2. during hyperinsulinemic euglycemic glucose clamp studies, a much lower glucose infusion rate maintained euglycemia at a clamped supraphysiological plasma insulin level in 'tolerant' than in 'non-tolerant'animals.

We conclude that acutely nitrate patches improve insulin sensitivity whereas a 7-day subchronic treatment schedule that results in hemodynamic nitrate tolerance also produces insulin resistance.

4.2. Study of the effect of nitrate tolerance and the potencial role of CCK of meal-induced insulin sensitization in conscious rabbits

Hemodynamic Nitrate Tolerance Achieved by Transdermal NG

Transdermal NG produced a significant decrease in mean arterial blood pressure after the first patch on, with no change in the heart rate. The third and the seventh patch on did not produce

any change in these parameters. Thirty minutes 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 mmHg 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 rabbits 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. Methemoglobin formation did not exceed 1 % over the study in either tolerant or intolerant animals.

Insulin Sensitivity Reflected in the RIST Index in Fasted and Refed Animals With and Without Nitrate Tolerance

The RIST index was almost doubled in animals with placebo patches reexposed to food as compared with their fasting littermates. In rabbits with established hemodynamic nitrate tolerance, however, reexposure to food was without effect on the RIST index. Fasting RIST values were lower in nitrate-tolerant animals than those in the intolerant ones.

Insulin Sensitivity Measured by HEGC in Fasted and Refed Animals With and Without Nitrate Tolerance

The glucose infusion rate needed to maintain euglycemia at clamped hyperinsulinemia increased with reexposure to food as compared with the corresponding fasting values in intolerant 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

The CCK8 increased the rate of glucose infusion necessary to maintain euglycemia 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 pmol/mg 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 intolerant and tolerant animals, respectively.

The results show that the state of hemodynamic nitrate tolerance achieved by a 7-day continuous exposure to transdermal NG is associated with the loss of the mealinduced 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 NG. An additional original observation is that the insulin-sensitizing effect of intraportal CCK8 is impaired in nitrate tolerance.

The present work demonstrates that the meal-induced insulin-sensitizing mechanism is seriously impaired in nitrate-tolerant rabbits. Whatever the method used, the results clearly demonstrate that whole-body insulin sensitivity is significantly increased when the animals are reexposed to food after a preceeding period of fasting and that this food-induced insulin sensitization phenomenon is blocked in nitrate tolerance.

CCK plays a key role in the activation of the postprandial insulin sensitization mechanisms. Proglumide, a CCK receptor antagonist, blocked the phenomenon of meal-induced insulin sensitization furthermore our results demonstrate that CCK has insulin sensitizing effect since the intraportal infusion of CCK8 produce a dose-dependent increase in whole-body insulin sensitivity, an effect also completely blocked by nitrate tolerance.

5. Summary

The insulin sensitizing effect of NO is strogly established and since it has been known that tolerance can developed against the hemodynamic effect of NO, we set an aim to study whether nitrate tolerance developes against the insulin sensitizing effect as well? Our results confirm that acute nitrate patches improve insulin sensitivity whereas a 7-day continuous (non intermittant) treatment schedule beside the hemodynamic nitrate tolerance also produces insulin resistance.

Since previous studies have shown the role of NO in the mechanisms (HISS) of meal induced insulin sensitivity and since the permanent impairement of HISS-mechanisms can lead to the developement of type 2 diabetes, we also studied how the developed hemodynamic nitrate tolerance can have an effect on the HISS-mechanisms? We conclude that in hemodynamic nitrate tolerance not only baseline, starving insulin resistance, but also postprandial insulin resistance developes. Moreover, we provide further evidence for the insulin sensitizing effect of CCK, we demonstrated that the NO-cGMP pathway plays a central role in the insulin sensitizing effect of CCK.

Taken together, our results support the original observation of Kovács et al., that the insulin senzitizing effect can be attained by using transdermal nitroglycerin patch. At the same time our results draws attention to importance of choosing correctly the nitrate therapy, because the nitrate therapy can be a double edged sword in the treatment of patients with ischemic heart failer, who frequently can have in the backround insulin resistance as a part of the metabolic syndrome.

6.Appendix

Candidate: Ágnes Bajza

Neptun ID: YDS8KF

Doctoral School: Doctoral School of Pharmaceutical Sciences

List of publications related to the dissertation

1. **Bajza, Á.**, Németh, J., Peitl, B., Szilvássy, Z.: Block by nitrate tolerance of meal-induced insulin sensitization in conscious rabbits.
   J. Cardiovasc. Pharmacol. 58 (5), 508-513, 2011.
   DOI: http://dx.doi.org/10.1097/FJC.0b013e31822bf556
   IF:2.287

2. **Bajza, Á.**, Peitl, B., Németh, J., Pórszász, R., Rablóczky, G., Literati-Nagy, P., Szilvássy, J., Szilvássy, Z.: Development of insulin resistance by nitrate tolerance in conscious rabbits.
   J. Cardiovasc. Pharmacol. 43 (3), 471-476, 2004.
   IF:1.576

List of other publications

3. Literáti-Nagy, Z., Tory, K., Literáti-Nagy, B., **Bajza, Á.**, jr. Vígh, L., Vígh, L., Mandl, J., Szilvássy, Z.: Synergetic Insulin Sensitizing Effect of Rimonabant and BGP-15 in Zucker-Obes Rats.
   Pathol. Oncol. Res. 12253, 1-5, 2013.
   DOI: http://dx.doi.org/10.1007/s12253-013-9620-6
   IF:1.555 (2012)

4. Zsuga, J., Tory, K., Jaszlits, L., **Bajza, Á.**, Németh, J., Peitl, B., Szilvássy, Z.: Pre-clinical methods for the determination of insulin sensitivity.
   J. Biochem. Biophys. Methods. 61 (1-2), 253-258, 2004.
   DOI: http://dx.doi.org/10.1016/j.jbbm.2004.06.006
   IF:1.302

H-4032 Debrecen, Egyetem tér 1. e-mail: publikaciok@lib.unideb.hu

5. Gesztelyi, R., Zsuga, J., Cseppentő, Á., **Bajza, Á.**, Varga, A., Szabó, J.Z., Szentmiklósi, J.A.: Special sensitization pattern in adenosine-induced myocardial responses after thyroxine-treatment. *J. Pharmacol. Sci. 91* (4), 295-304, 2003.
DOI: http://dx.doi.org/10.1254/jphs.91.295

Total IF of journals (all publications): 6.72

Total IF of journals (publications related to the dissertation): 3.863

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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