General and Comparative Endocrinology 183 (2013) 69–78

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Development of hyperglycemia and diabetes in captive Polish bank voles

Aleksandra Bartelik [a,b,1], Maciej Ciesla [a,1], Jerzy Kotlinowski [a], Stanislaw Bartelik [c], Dominik Czaplicki [d], Anna Grochot-Przeczek [a], Krzysztof Kurowski [e], Paweł Koteja [f], Jozef Dulak [a], Alicja Józkowicz [a,*]

[a] Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
[b] Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
[c] Regional Center of Blood Donation and Haemotherapy, Kielce, Poland
[d] Laboratory of Molecular Genetics and Virusology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
[e] R&D Department, Adamed Ltd., Pienkow, Poland
[f] Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland

ARTICLE INFO

Article history:
Received 5 November 2011
Revised 14 December 2012
Accepted 18 December 2012
Available online 3 January 2013

Keywords:
Bank vole
Diabetes
Postnatal stress
Glycemia
Insulin
Family history of diabetes

ABSTRACT

Diabetes has been detected in Danish and Swedish bank voles (*Myodes glareolus*). There are no data, however, concerning the prevalence of diabetes in populations from other geographic regions. We investigated the frequency and physiological effects of glucose metabolism disorders in captive bank voles from Poland.

Single measurement of fasting blood glucose concentration performed in the 3–4 month old captive-born bank Polish voles without any disease symptoms showed that 8% of individuals (22/284) displayed an impaired fasting glucose (IFG, blood glucose (BG) $\geqslant$100 mg/dL) and 1% (4/284) showed hyperglycemia (BG $\geqslant$ 126 mg/dL) which could suggest diabetes. Next, we analyzed blood glucose in samples taken once a month from an additional cohort of bank voles with (FHD), or without (H), a family history of diabetes. The prevalence of IFG at age six months was 26% (16/62) among bank voles from the H group. In the FHD group the prevalence increased to 49% (43/88), and additional 12% (11/88) became diabetic (DB, BG $\geqslant$ 126 mg/dL at two time points). Postnatal stress (three maternal deprivations before weaning) did not affect the risk of developing IFG or DB in H voles, but significantly reduced the frequency of glucose metabolism disorders (IFG and DB combined) in FHD voles. IFG was associated with hyperinsulinemia, but not with other biochemical disturbances. Diabetic animals displayed a progressive malformation and vacuolization of β-cells in the pancreas, without visible leukocytic infiltrations. In summary, our results indicate that Polish captive bank voles can develop diabetes, which shows features of both type 1 and type 2 diabetes in humans. Risk of diabetes is higher in animal with FHD.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by elevated serum glucose level, accompanied by many non-specific symptoms, such as polyuria or polydipsia [18]. Type 1 diabetes (T1DM) results from decrease or lack of insulin production due to autoimmune destruction of β-cells [1]. Type 2 diabetes (T2DM), the most frequent type of diabetes, is preceded by a progressive insensitivity to insulin [61]. Insulin resistance is usually followed by insufficient insulin secretion due to exhaustion and death of β-cells, which later results in complete lack of endogenous hormone [11].

The autoimmune response leading to T1DM can be influenced by genetic susceptibility and environmental factors like stress, diet, or viral infections [43]. Possible contribution of enteroviruses, retroviruses, coxsackie viruses, rubella viruses, cytomegaloviruses, or Epstein–Barr viruses in induction of diabetes in animal models and human populations has been repeatedly suggested [26,45,60]. Viruses can trigger destruction of insulin-producing cells in animals, acting via different mechanisms, including a direct β-cell lysis, bystander activation of autoreactive T cells, loss of regulatory T cells, and molecular mimicry [57]. On the other hand, stress is supposed to precipitate diabetes, increase the insulin resistance and trigger or promote the progress of diabetes-related autoimmunity [51]. Accordingly, it was demonstrated that psychological stress in human infants is associated with significantly increased frequencies of T1DM [27]. Also in the BB rats, the model of T1DM, psychological stress such as restraint, rotation, and crowding, independent of weight gain or food intake, significantly reduced the average age of onset and increased the prevalence of diabetes [16].

The most commonly used models of diabetes are mice or rats injected with streptozotocin (STZ) or alloxan. Depending on the dose and time-course of treatment the generated pathology

* Corresponding author. Address: Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland. Fax: +48 12 664 6918.
E-mail address: alicja.jozkowicz@uj.edu.pl (A. Józkowicz).
[1] These authors contributed equally to this work.

0016-6480/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2012.12.006

resembles T1DM or T2DM [46]. The genetic models include NOD (Non-obese Diabetic), db/db, ob/ob, and NZO (New Zealand Obese) mice or BB, ZDF (Zucker Diabetic Fatty), and Goto-Kakizaki rats. These strains demonstrate various degrees of hyperglycemia and insulinemia, accompanied or not by obesity [30,44]. Some models of diabetes have been developed after observation that animals taken from their natural environment develop diabetes mellitus in laboratory. Among these are the sand rat (*Psammomys obesus*), the spiny mouse (*Acomys cahirinus*), the tuco-tuco (*Ctenomys talaru*), the Chinese hamster (*Cricetulus griseus*), the Djungarian hamster (*Phodopus sungorus*), and the South African hamster (*Mystromys albicaudatus*) [34,53].

Studies performed in Danish populations of bank voles (*Myodes glareolus*) showed that approximately 20% of captive individuals displayed polydipsia, accompanied by glucosuria [18,48]. Moreover, the development of polydipsia was associated with progression of typical symptoms of T1DM, such as hyperglycemia, glucosuria, ketonuria, ketonemia, and destroyed β-cells [36]. Individuals showing high blood glucose levels and impaired glucose tolerance were also found among the bank voles originating from Sweden, trapped at a local population peak of medium density [37]. Although autoantibodies and the β-cell destruction were not seen in wild animals, the diabetic symptoms did develop in some of them over a time period of two months in captivity [37]. Interestingly, two reports indicated a role of Ljungan virus (LV) in destruction of β-cells, the picornavirus detected in the bank voles from Denmark and Sweden [36,37], and from northern Italy [24]. LV has also been found in montane voles (*Microtus montanus*), and red-backed voles (*Myodes gapperi*) from the USA [55]. It has been even hypothesized that the bank vole might be a vector for this pathogen, increasing the risk of T1DM in humans [40]. However, RNA from LV has not been found in human T1D [35], and all independent studies have failed to establish a connection between LV and human T1D [54].

Captive Danish bank voles have been suggested as a lean model of diabetes, specifically T1DM [49]. On the other hand, captive individuals originating from Swedish population had elevated serum glucose and insulin levels, and the development of glucose intolerance led to diabetes with features of both T1DM and T2DM [6,8]. There are no data, however, concerning the prevalence and characterization of diabetes in the bank voles from other geographic regions. Therefore, the aim of our study was to investigate the frequency and physiological effects of glucose metabolism disorders in captive bank voles from Poland.

Analyses done in diabetes-prone Danish voles demonstrated that probability of T1DM development in adulthood can depend on postnatal stress [18]. Two types of stressors were tested: low- and high-frequent maternal deprivation, which turned-out to be ineffective, and immersions in luke-warm water, which influenced the later proneness to develop T1DM, but in both directions – either increasing the prevalence of diabetes when applied as a low-frequent stress or decreasing it, when applied as a high-frequent stress [18]. In our study we investigated whether postnatal low-frequent stress (maternal deprivation) may influence development of diabetes in Polish captive bank voles. We compared the reaction of animals from subgroups with or without family history of diabetes.

2. Materials and methods

2.1. Animals

All experiments were performed in animal facility of the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University. Breeding pairs were received from animal facility of the Institute of Environmental Sciences of the Jagiellonian University [28,47]. This colony was reared from about 320 animals originally captured in the field in Niepołomice Forest (southern Poland) in Summer/Autumn 2000 and 2001. Approximately two generations per year were produced, with more than 100 reproducing animals in each generation. The study was performed in 2006 and 2007, i.e. the animals used as parents represented 7–10th laboratory-bred generation (because the colony included animals captured on two years, and because in one year the breeding included animals from overlapping generations, the exact generation number cannot be specified [47]).

Young bank voles selected for experiments were separated from mothers at the day 30th and housed individually. Dimensions of cages were 20 × 26 × 14 cm, and 28 × 43 × 16 cm for individually housed voles and breeders, respectively. Animals were maintained under controlled environmental conditions (12L:12D photoperiod with light turned on from 7.00 a.m. to 7.00 p.m., temperature of approximately 20 °C), in standard laboratory mouse cages with a wood cutting bed, under a minimum of extraneous disturbance. Cages were changed every 10–14 days. In mothers with puppies cages were changed for the first time at the day 10th after parturition. Standard laboratory food (Labofeed H, Kcynia) and water were available *ad libitum*. Food contained 25% of protein, 6% of fat, 5% of fibers, and was supplemented with vitamins (A, D_3, E, K_3, B_1, B_2, B_6, B_9, B_{12}). All animal protocols were accepted by the Animal Care and Use Committee at the Jagiellonian University.

531 bank voles (304 ♀ and 227 ♂) were used in the research, including 284 individuals (161 ♀ and 123 ♂) at the age of 3–4 months for the primary measurements of blood glucose in the colony studied, and 247 individuals (143 ♀ and 104 ♂) at the age of 1–6 months in the experiment on effect of stress and inherited factors on development of diabetes. Each animal has participated in only one part of the research.

2.2. Effect of stress and inherited factors on development of diabetes

The pedigrees and health status of ancestors for all animals have been documented for at least three generations. To check whether postnatal stress or inherited factors may influence the risk of development of diabetes, the mating pairs of voles without any signs of disease, were divided into two groups based on the symptoms of diabetes mellitus (polydipsia and polyuria [49]) found, relying on the judgments noticed in animal inspection records, in at least one of their parents or grand-parents: (i) 30 pairs of animals with no symptoms noted in the ancestors (H); ii) 43 pairs of animals with a family history of diabetes (FHD), where at least one parent or grand-parent to each of the mating partners had shown symptoms of diabetes. The experiment was performed in the first-born generation, namely the pups from the first litters (L) of these breeding pairs. To estimate a role of postnatal stress, randomly selected puppies were separated from mothers for 4 h on days 5th, 10th, and 15th, following a modification of the protocol described by Freimanis et al. [18]. We chosen this modified schedule to adjust to time of first changes of cages in mothers with puppies, which were performed at day 10th after parturition. Randomization was performed after the litters were born. The resulting subgroups were named (i) H-NS – with healthy ancestors, non-stressed ($N = 62$, 31 ♀, 31 ♂, 19 L); (ii) H-S – with healthy ancestors, stressed ($N = 39$, 22 ♀ and 17 ♂, 11 L); (iii) FHD-NS – with FHD, non-stressed ($N = 88$, 55 ♀ and 33 ♂, 26 L); and (iv) FHD-S – with FHD-stressed ($N = 58$, 35 ♀ and 23 ♂, 17 L). Only animals which survived for 6 months ($N = 247$) were taken for analyzes, to monitor the changes of glucose concentration in the same individuals. Six month survival rate was 83–84% in all groups.