Rapid communication

Promotion of diabetes onset by stress in the BB rat

W.R. Carter, J. Herrman, K. Stokes and D.J. Cox

Department of Behavioral Medicine and Psychiatry, University of Virginia School of Medicine, Charlottesville, Virginia, USA

Summary. The purpose of the present study was to address the question of whether environmental stress affects the age of onset and incidence of overt insulin-dependent diabetes mellitus in an animal model of human Type 1 (insulin-dependent) diabetes. Thirty-five diabetes prone BB rats of both sexes were divided into two groups: experimental animals were exposed to multiple, concurrent, and unpredictable en-

vironmental stressors, while littermate controls were exposed to a standard lab environment. Groups were housed in two separate, sound attenuated, independently ventilated, and restricted rooms. By age 150 days, experimental group animals had developed overt diabetes at a significantly earlier age.

Key words: Stress, type, diabetes, onset.

The question of whether overt onset of human Type 1 (insulin-dependent) diabetes may be modified by environmental stress has not been fully addressed by experimental investigation. However, it is generally accepted that Type 1 etiology involves a combination of genetic susceptibility and environmental factors, such as infection, etc. [1]. We know that immune system response to acute infection, as well as to chronic disease, can be modified by environmental stress [2]. Moreover, Type 1 diabetic patients often report that onset of clinical manifestations of their diabetes was preceded by some stressful or traumatic event [3, 4]. However, retrospective patient report is subject to a multitude of intervening variables, such as effect of patient recall and bias in assignment of meaning to otherwise unrelated life events.

In earlier animal investigations of this question, "vulnerability" to insulin dependent diabetes was attained through B-cell lesion either by surgery [5] or streptozotocin [6]. Subsequent administration of stress to lesioned animals in these studies resulted in either an increased [5] or decreased [6] incidence of overt diabetes. The reason for these mixed results may have been the lack of a more appropriate animal model of the diabetes process.

A more appropriate animal model to address the question of the effect of stress on Type 1 diabetes onset may be the diabetes prone BB rat [7]. By 150 days of age, 30 to 70% of these animals, depending on subline, exhibit impaired glucose tolerance, hypoinsulinaemia, lymphopenia, and, with onset, hyperglycaemia with hypergluconaeamia, hyperphagia, polydipsia, polyuria and glycosuria [8], and peripheral neuropathy [9]. Mean age of onset of overt clinical diabetes ranges from 60 to 110 days, depending on subline. If insulin is not administered, animals exhibit preterminal declines in weight and physical activity. Appearance of diabetes

in the BB rat has been successfully decreased [10] and increased [11] primarily via immunologic intervention [10].

In order to address the question of whether environmental stress mediates the appearance of diabetes in a more appropriate animal model of human Type 1 diabetes, we report a study in which diabetes-prone BB rats were exposed to multiple, concurrent, and unpredictable psychological stressors. Dependent variables were incidence of overt diabetes and average age of onset in experimental and control groups.

Materials and methods

Animals

Thirty-five 30-day-old diabetes prone BB Wistar rats were obtained from the University of Massachusetts BB rat colony and housed in standard single-animal cages. Animals were maintained at 23°C room temperature, a standard 12-h light-dark cycle, and ad libitum food and Polyotic (American Cyanamide Co., Wayne, NJ, USA) tetracycline hydrochloride. Animals were weighed daily. Weight of consumed food was determined every 4 days by weighing remaining food and subtracting from standard amount provided.

Procedure

At 54 days, littermates were divided into stressed or experimental ($n=25$; 10 male) and control ($n=10$; 3 male) groups, each in two independently ventilated rooms. Experimental animals were housed in separate group cages and received the concurrent administration of stressors described below. Urinary glucose and ketone levels were determined daily using Lilly Tes-Tape. Rats were determined to exhibit overt diabetes by exhibiting 2 consecutive days of glycosuria followed by measurement of venous blood with reagent strip and reflectance meter. Urine and blood glucose was measured preceding stressor onset. Urine was obtained by applying abdominal pressure in both groups. At determination of diabetes or at age 150 days, animals exited the study. All animals exiting the study due to diabetes required immediate daily insulin injections; absence of this treatment resulted in death.

Fig. 1. Incidence of diabetes in stressed (—★—) and control (—●—) animals

Experimental stressors

The following stressors were administered simultaneously to experimental animals.

Restraint. Animals were placed in plastic restraining cages which prevented movement, without physical pressure, 1 h three times per week at random times.

Rotation. Each group cage was mounted on equipment which rotated the cage at 33 rpm. 15 min rotations occurred automatically and randomly every 45 to 150 min for 24 consecutive hours every other day. Since the maximum gravitational force on the animals is less than 1 g at 33 rpm, it was not centrifugal.

Crowding. Experimental animals were housed in group cages (23 × 46 × 20 cm) of 5 to 8 rats each, depending on animal weight, while control animals remained in individual cages placed side-by-side for the duration of the study.

Resocialization. Every 3 days, experimental animals were randomly reassigned to different group cages. This manipulation denied the rats the opportunity to establish a social dominance order, requiring constant ritualized aggressive and aggression-avoidance behaviors.

Statistical analysis

The first dependent measure was age (day) of first glycosuria if it occurred on two consecutive days. The second dependent measure was group incidence of overt diabetes by age 150 days.

Results

Age of onset of overt diabetes is plotted against cumulative incidence rate for stressed and control groups in Figure 1. Stressed BB rats exhibited a significantly earlier age of onset than littermate controls as determined by Student's *t* test ($t=2.28$, $p<0.05$, twotailed). Although inspection of data plotted in Figure 1 suggests a higher incidence of diabetes in stressed animals than in controls, chi square analysis of this trend was not significant.

In order to examine the possible effect of food consumption and weight gain on these findings, between-groups analyses of food consumption and weight gain

were conducted. Univariate analyses of variance of these data indicated neither weight gain nor food consumption differed between the two groups.

Discussion

Results of this study indicate that environmental stress may modify age of onset of overt diabetes in the diabetes prone BB rat. The effect of stress on diabetes onset was found to be unrelated to weight gain or food consumption. The stressors utilised in this study were multiple, concurrent, relatively strong, changing, and unpredictable.

These findings are preliminary and require replication. Hence, the actual degree of effect of stress on diabetes onset in the BB rat is currently unclear. However, these findings may relate to retrospective [3, 4] and informal clinical reports of stressful life events preceding the appearance of overt human Type 1 diabetes. Whether these findings can be directly related to the expression of human Type 1 diabetes awaits further investigation.

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Received: 15 June 1987

Dr. W. R. Carter
Department of Behavioral Medicine and Psychiatry
University of Virginia School of Medicine
Charlottesville, VA 22901, USA