Short Communication

The number of lactotrophs is reduced in the anterior pituitary of streptozotocin-induced diabetic rats

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Abstract

Aims/hypothesis. Prolactin secretion is often reduced in insulin dependent diabetes mellitus, but little is known about the mechanism involved. Since changes in the hormonal environment modulate cell proliferation, death and cellular makeup of the anterior pituitary, we have analysed whether the number of lactotrophs is reduced in diabetic rats.

Methods. Streptozotocin induced diabetic rats were maintained hyperglycaemic for 2 months. Pituitary prolactin, growth hormone, Bcl-2, Bax and PCNA concentrations were analysed by western blot analysis. In situ hybridisation was used for quantification of prolactin and growth hormone mRNA containing cells. Cell death was detected by TUNEL labelling, alone and in combination with immunocytochemistry for prolactin or growth hormone.

Results. Diabetic rats had fewer lactotrophs ($p<0.01$). This was coincident with a decrease in overall protein and prolactin content. An increase in pituitary cell death was found and some of the TUNEL labelling co-localised with prolactin immunostaining. No change in the concentration of Bcl-2 or Bax, proteins implicated in apoptosis, was detected. PCNA content was higher in the pituitaries of diabetic rats, suggesting increased proliferation.

Conclusion/interpretation. Anterior pituitary cell turnover is affected in poorly controlled diabetes mellitus. A decrease in the number of lactotrophs, as a result of increased cell death, could underlie, at least in part, the reduction in prolactin secretion observed in diabetic animals. [Diabetologia (2003) 46:634–638]

Keywords Lactotroph, prolactin, somatotroph, growth hormone, pituitary, cell turnover, apoptosis, Bcl-2, Bax, proliferation.

In poorly controlled diabetic patients or laboratory animals, circulating prolactin (PRL) concentrations and PRL responses to secretory stimuli are often suppressed [1, 2, 3]. This results from a decrease in pulse amplitude, peak area and interpulse concentrations,

but not pulse frequency [1]. Furthermore, the secretory activity of individual lactotrophs is not impaired in diabetic rats [3], suggesting diminished stimulatory input to these cells. However, a decrease in the number of lactotrophs could also explain a reduction in PRL output, with no loss in synthetic capacity of individual lactotrophs. The anterior pituitary undergoes cellular turnover during adulthood and this is affected by changes in the hormonal environment [4]. Hence, the cellular makeup of the anterior pituitary could be affected in diabetes mellitus. We have analysed whether the number of lactotrophs is modulated in poorly controlled diabetic rats and the possible mechanisms involved in this process.

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Abbreviations: PRL, prolactin; GH, growth hormone; PCNA, proliferating cell nuclear antigen; TUNEL, TdT-mediated dUTP nick-end labelling.

Materials and methods

Animals. Adult male Wistar rats were injected (i.p.) with 65 mg/kg streptozotocin (Sigma, Steinheim, Germany) and control rats received vehicle. Blood glucose concentrations were measured via tail puncture (Glucocard Memory 2; Menarini Diagnostic, Florence, Italy) to verify the diabetic state. Two months later, the rats were decapitated and the pituitaries removed and frozen on dry ice.

The rats were treated according to the European Community laws for animal care.

Immunoblotting. Immunoblotting was carried out [5] and the protein concentration was estimated by Bio-Rad protein assay (Bio-Rad, Hercules, Calif., USA) and 10 µg was resolved using 12% SDS-PAGE and transferred to PVDF membranes (Bio-Rad). Filters were incubated with 1° antibody (GH and PRL 1:5000, National Hormone and Pituitary Program; Bcl-2 and Bax 1:200, Neomarkers, Fremont Calif., USA; PCNA 1:2500, Sigmanet, Denham, Mass., USA), washed and incubated with 2° antibody conjugated with peroxidase or biotin (Pierce, Rockford, Ill., USA). Peroxidase activity was visualised by chemiluminescence (Dupont-NEN, Boston, Mass., USA) and quantified by densitometry (Bio-1D, Vilber Lourmat, Marne La Vallee, France).

Results are expressed as the amount of the substance of interest per µg protein. As total protein per pituitary was reduced in diabetic rats, the amount of substance of interest per pituitary was calculated (e.g., µg protein/pituitary × PRL/µg protein). The amount of GH or PRL produced per cell might not change, but modulation of total hormone per pituitary would influence serum concentrations. This could be of less importance for substances that act locally, such as Bcl-2 or Bax.

In situ hybridisation. In vitro transcription of digoxigenin riboprobes was carried out according to the manufacturer's instructions (Roche, Barcelona, Spain). The riboprobes, in situ hybridisation methodology and pertinent controls have been described previously [6].

Quantification of cell number. The number of GH mRNA or PRL mRNA containing cells per area was calculated by analysing six tissue sections per rat. Using a 100× objective, the number of positive cells in 12 fields per section (12 fields/section, 6 tissue sections/pituitary or 72 fields/pituitary) was analysed. Analysis was done twice by two different operators. The error of repetition of each operator and between operators was less than 10%.

Cell death assessment. Cell death detection assays were done according to the manufacturer's instructions (Roche). Fluorescein-16-deoxy-UTP was used and TUNEL signal was visualised with a fluorescent microscope (Leica, Madrid, Spain).

Immunohistochemistry/TUNEL. Sections were processed for TUNEL labelling and then PRL or GH antibody (1:5000) in PBS containing 3% BSA and 1% Triton X-100 was applied and left overnight at 4°C. Sections were incubated with a biotin-conjugated 2° antibody (Pierce; 1:1000) for 1.5 h and streptavidin-Alexa Fluor 594 conjugate (Molecular Probes, Eugene, OR; 1:2000) for 1 h. Signal was visualised with a confocal microscope (Leica, Madrid, Spain).

Statistical analysis. Data are expressed as means ± SEM and were analysed by two-tailed Student's *t*-test. A *p* value of less than 0.05 was considered statistically significant.

Results

Protein content. In poorly controlled diabetic rats there was a reduction in total protein per pituitary compared to control rats (control: 1782±387 µg/pituitary vs 963±81 µg/pituitary; *p*<0.05; *n*=6).

PRL and growth hormone concentrations. Mean PRL per µg protein in diabetic rats was not different from the control rats (Fig. 1A). However, total PRL per pituitary was lower in diabetic rats (Fig. 1B; *p*<0.01).

Fig. 1. (A) Mean prolactin concentrations in control and diabetic rats (*n*=6) as measured by western blot analysis, expressed as prolactin/µg total protein. There was no significant difference between groups. (B) Relative amounts of total prolactin/pituitary (mean densitometric values × total protein/pituitary). Diabetic rats had less total prolactin per pituitary compared to controls (*n*=6, *p*<0.01). (C) The mean number of prolactin mRNA containing cell/unit area in control and diabetic rats. Diabetic rats had fewer detectable prolactin positive cells than control animals (*n*=3, *p*<0.01). (D) Mean growth hormone (GH) concentrations in control and diabetic rats (*n*=6) as measured by western blot analysis expressed as GH/µg total protein. There was no significant difference between groups. (E) Relative amounts of total GH/pituitary (mean densitometric values × total protein). There was no significant difference between groups in the total amount of GH per pituitary (*p*<0.05). (F) The mean number of growth hormone mRNA containing cell/unit area in control and diabetic rats (*n*=3). No significant difference was found. ■ control, □ diabetic, NS not significant

Fig. 2 A–E. (A) In the pituitaries of diabetic rats, aggregates of TUNEL positive cells (*green*) were observed. (B) TUNEL labelled cells could also be found dispersed throughout the pituitary of diabetic rats. (C) When sections were labelled for both growth hormone (*red*) and TUNEL (*green*) no double-labelled cells were detected. (D) Cells double-labelled for both prolactin (*red*) and TUNEL (*green*) could be found isolated in the pituitary, but they were rare. (E) In the clusters of TUNEL positive cells (*green*), some were found to also label for prolactin (*red*). Arrows represent some double labelled cells

GH per μg protein did not differ between diabetic rats and control rats (Fig. 1D). Mean GH per pituitary tended to be less in diabetic rats, but this was not statistically significant (Fig. 1E).

Number of PRL and GH mRNA containing cells per area. The number of PRL mRNA containing cells/area was decreased in diabetic rats compared to control rats (Fig. 1C). There was no difference in the number of GH mRNA containing cells per area (Fig. 1F).

TUNEL labelling. TUNEL labelling was increased in pituitaries of diabetic rats (Fig. 2A,B). These cells tended to be grouped (Fig. 2A), but some were dispersed throughout the pituitary (Fig. 2B). No control data is represented as no clusters of cells and few or no dispersed cells were seen.

Somatotrophs were clearly labelled, but no GH immunostaining was found in TUNEL-labelled cells (Fig. 2C).

Immunostaining for PRL in conjunction with TUNEL revealed double-labelled cells in diabetic animals (Fig. 2D,E), but not in control rats.

Bcl-2 and Bax protein concentrations. No difference in mean concentrations of the anti-apoptotic protein Bcl-2 (control: 95.7 ± 20.4 DU vs diabetic: 107.7 ± 31.4 DU; $n=4$) or the pro-apoptotic protein Bax (control: 69.9 ± 10.7 DU vs diabetic: 64.2 ± 6.5 DU; $n=4$) were found. These values were not different between groups even after normalisation for total protein content of the pituitary.

Cell proliferation. Diabetic rats had higher concentrations of PCNA than control rats (control: 36.7 ± 7.7 DU vs diabetic: 82.5 ± 9.0 DU; $p < 0.001$; $n=5$).

Discussion

In poorly controlled diabetic patients or animals PRL concentrations are often decreased [1, 2, 3]. PRL is involved in lactation and diabetic women could have reduced and/or delayed milk production [7]. Milk production is reduced in streptozotocin-induced diabetic rats and this is correlated with a decrease in suckling-induced PRL secretion [2]. However, the underlying mechanism remains unknown.

Total protein, PRL content and the number of lactotrophs are reduced in streptozotocin-induced diabetic rats. The decrease in protein content is most likely due to the reduction in total cell number. A decline in the mean PRL mRNA content per cell could result in more cells below the limit of detectability. However, the increase in PRL immunopositive-TUNEL positive cells indicates increased lactotroph death. No change in the apoptotic proteins Bcl-2 or Bax suggests death

occurs via another apoptotic pathway or mechanism. Indeed, pituitary cell death after interruption of lactation does not occur by apoptosis [8, 9]. In contrast, in males or ovariectomized females, apoptosis in the pituitary can be induced by bromocryptine [9], indicating protection from apoptotic cell death by sex steroids.

GH concentrations are decreased in diabetic rats [3, 10]; however, there is no change in the number of somatotrophs per area, TUNEL labelled somatotrophs or pituitary GH content was found, although GH mRNA concentrations are decreased in this paradigm [10]. A decrease in both production and secretion could result in no change in static GH protein content. Somatotroph turnover could be less responsive in this paradigm as lactotrophs are more sensitive to changes in the hormonal environment than other pituitary cell types [4].

Proliferation was also increased in pituitaries of diabetic rats. Since PCNA was measured in total pituitary extracts, the cell type involved cannot be verified. However, our data indicate that there is an increase in cell turnover, both proliferation and cell death, in the pituitaries of streptozotocin-induced diabetic rats and the number of lactotrophs is decreased. The caveat that streptozotocin itself may affect pituitary cell turnover must be considered. However, the hormonal changes seen in this model are similar to that in other animal models and human diabetic patients. Hence, the decrease in the total number of lactotrophs in diabetic rats could be responsible, at least in part, for the decrease in prolactin production.

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