Prolactin protects against diabetes induced by multiple low doses of streptozotocin in mice

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Abstract

In earlier studies it has been shown that prolactin (PRL) is a stimulating factor for the immune system, and it has been suggested that PRL might antagonize immunosuppressive effects of glucocorticoids. PRL has been reported to affect the cytokine secretion pattern, by elevating cytokine gene expression in macrophages, after the onset of sepsis. It also promotes the antibody response in mice where it increases the production of interferon-γ (IFN-γ) and inhibits interleukin-1 (IL-1) production. Due to these properties, PRL might influence the development of autoimmune type 1 diabetes. The aim of the present study was to examine the effects of two drugs; PRL and bromocriptine (BC) in vivo on the development of hyperglycemia and pancreatic insulitis in mice treated with multiple doses of streptozotocin (STZ) (40 mg/kg body weight, i.p.). The dopaminergic agonist BC is known to inhibit PRL secretion. In another set of experiments, the direct effects of PRL on the function of pancreatic islets exposed to STZ in vitro were studied. Mice treated with STZ became gradually hyperglycemic, and concomitant treatment with PRL (4 mg/kg body weight) for 21 days significantly reduced the elevation in blood glucose levels from day 10 onwards (P<0·05). Morphologic examinations of the pancreas on day 21 of mice receiving STZ injections revealed a marked insulitis, but only moderate insulitis in the STZ treated animals given PRL. BC administration (10 mg/kg body weight) in combination with STZ did not significantly affect the elevation in blood glucose levels or the insulitis. PRL or BC administration alone did not change the serum glucose concentration. This study indicates that PRL may affect hyperglycemia in the early phase of autoimmune diabetes. We suggest that it might be due to counteraction of autoimmune immunologic mechanisms and/or enhancement of β-cell regeneration.

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Introduction

Prolactin (PRL), a neuroendocrine peptide hormone (24 kDa single structure) produced by the pituitary gland, has been suggested to play an important role in the regulation of the immune system because it stimulates lymphocyte proliferation and macrophage function (Spangelo et al. 1987, Gala 1991). For example, PRL has been reported to cause a significant elevation of cytokine gene expression in macrophages, after the onset of sepsis (Zhu et al. 1997). The stimulatory effect of PRL on immunity may result from antagonism of the immunosuppressive glucocorticoid effects (Berczi 1997). PRL promotes the antibody response and increases the production of interferon-γ (IFN-γ) and inhibits interleukin-1 (IL-1) production (Berczi 1997). A severe inflammation often results in elevated levels of circulating glucocorticoids and adrenocorticotropic (ACTH), which in turn, reduces the secretion of PRL (Bateman et al. 1989, Parrott & Goode 1993). Administration of PRL, under these circumstances, might therefore influence the progression of the inflammatory response. Moreover, several experimental studies and clinical observations have suggested that PRL might be important in the pathogenesis of various autoimmune diseases (Ferrari et al. 1983, Berczi 1993, Walker et al. 1993, Neidhart 1998, Velkeniers et al. 1998), but very few studies have focused on the role of PRL in diabetes mellitus type 1. It is therefore interesting to study whether PRL affects diabetes induced by multiple low doses of streptozotocin (STZ), a well established animal model for the pathogenesis of type 1 diabetes (Like & Rossini 1976, Kolb-Bachofen et al. 1988).

The aim of the experiments in the present study was to examine the effects of two drugs; PRL and bromocriptine (BC) in vivo on the development of hyperglycemia and pancreatic insulitis in mice treated with multiple intra-peritoneal injections of STZ. The dopaminergic agonist BC is known to inhibit PRL secretion (Sinha et al. 1976). In another set of experiments, the direct effects of PRL on the function of pancreatic islets exposed to STZ in vitro was studied. Previous data have shown that PRL
stimulates insulin secretion and proliferation of β-cells in islets of murine and human origin (Brelje et al. 1993, Sorensen et al. 1993).

Materials and Methods

Animals and treatment

Inbred adult male C57BL/KsJ mice, aged 3–4 months, weighing about 30 g, were used. These mice were originally obtained from the Jackson Laboratory (Bar Harbor, ME, USA) but they have been locally bred at the Biomedical Centre, Uppsala, Sweden for more than 18 years. The animals had free access to tap water and pelleted food throughout the experiments.

The mice were allocated to one of eight different groups of treatment: saline + saline, saline + vehicle, saline + PRL, saline + BC, STZ + saline, STZ + vehicle, STZ + PRL, STZ + BC. PRL (luteotropic hormone from sheep pituitary glands) and STZ were purchased from Sigma Chemicals (St Louis, MO, USA) and BC was kindly provided by Novartis (Ann Lindquist, Taby, Sweden). PRL was dissolved in saline, and for BC a vehicle composed of ethanol (70%):saline (1:9) and tartaric acid (54 mM) was used. The mice received either an intraperitoneal injection of saline (0·2 ml) or of STZ (40 mg/kg body weight; 0·2 ml). STZ was dissolved in cold citrate buffer (10 mM, pH 4·5). After 30 min the mice were given a second intraperitoneal injection of either saline, vehicle or of PRL (4 mg/kg body weight; 0·2 ml) or BC (10 mg/kg body weight; 0·2 ml). The first type of injection (saline or STZ) was given daily for 5 consecutive days, whilst the second type of injection (saline, vehicle, PRL or BC) was given daily until the animals were killed.

Blood glucose determinations (ExactTech blood glucose meter, Baxter Travenol, Deerfield, IL, USA) were performed on day 0, before any injection and on days 3, 7, 10, 14, 17 and 21. The blood samples were obtained from a tail vein of non-fasted mice.

Morphologic examination

The mice were killed by cervical dislocation and their pancreatic glands were removed and fixed in 10% formalin solution and embedded in paraffin. Sections 7 µm thick were cut and stained with hematoxylin and eosin. The pancreatic islet histology was ranked according to four arbitrary classes as previously described and illustrated (Jansson & Sandler 1988, Holstad & Sandler 1993); class A: normal islet structure, class B: mononuclear cell infiltration in the periphery of the islets, class C: infiltration of mononuclear cells into a majority of the islets i.e. insulitis, class D: only a few islets present often with altered structure, e.g. pyknotic cell nuclei.

The pancreatic sections were evaluated by two independent examiners being unaware of the origin of the sections.

Islet isolation, culture and incubation

Pancreatic islets were isolated from overnight fasted adult C57BL/KsJ mice by collagenase digestion (Collagenase A, Boehringer-Mannheim, Mannheim, Germany), as described in detail elsewhere (Sandler et al. 1987). The islets where then picked by hand, using a braking pipette. Groups of 150–200 islets were precultured free-floating for 6–7 days at 37 °C in an atmosphere of humidified air + 5% CO₂ in 5 ml medium RPMI 1640 containing 11·1 mM glucose (Sigma) supplemented with 10% (v/v) fetal calf serum (FCS) (Sigma). Medium was changed every second day. After the preculture period, islets in groups of 50 were transferred to new culture dishes containing 1 ml Krebs-Ringer bicarbonate buffer+Hepes (KRBH; 114·3 mM NaCl, 4·74 mM KCl, 1·15 mM KH₂PO₄, 1·18 mM MgSO₄, 25·0 mM NaHCO₃, 10·0 mM Hepes, 4·26 mM NaOH, 2·54 mM CaCl₂; pH 7·4) supplemented with 2 mg/ml bovine serum albumin (BSA; fraction V; ICN Biochemicals, Aurora, OH, USA) and 5·6 mM glucose with or without addition of PRL (6 or 60 µg/ml). The islets were incubated at 37 °C in a gas atmosphere of 95% O₂+5% CO₂ for 30 min. Then, STZ, dissolved in 0·9% NaCl, was added to the incubation medium (final concentration 1·8 mM STZ) for 30 min. After rinsing and removal of the medium the islets were cultured overnight in RPMI 1640 supplemented with 10% FCS.

Islet glucose-stimulated insulin release, insulin and DNA content

After the culture period, triplicate groups of 10 islets were transferred to sealed glass vials containing 250 µl KRBH supplemented with 2 mg/ml BSA and 1·7 mM glucose during the first hour. The islets were incubated at 37 °C in a gas phase of 95% O₂+5% CO₂. The incubation medium was then gently removed and replaced by KRBH and 2 mg/ml BSA supplemented with 16·7 mM glucose during the second hour. After the incubations, the islets were harvested and pooled in groups of 30 and homogenized in 0·2 ml redistilled water. A fraction of the homogenate was mixed with acid ethanol and insulin was extracted overnight at 4 °C. The insulin concentration of the extract was measured by RIA (Heding 1972). Another fraction of the aqueous homogenate was used for measurement of DNA content (Hinegardner 1971).

Statistical analysis

The blood glucose values are expressed as means ± s.e. and groups of data were compared using Student’s unpaired t-test. In the in vitro experiments, where islets were
incubated in triplicate, a mean was calculated for each experimental group and considered as one separate observation. Furthermore, every islet observation represents different mouse islet donors and values were expressed as means ± s.e. and groups of data were compared using Student’s paired t-test.

Results

Effects of PRL and BC on multiple low dose STZ-induced diabetes

The mice treated with multiple low doses of STZ became gradually hyperglycemic (Fig. 1). It was found that treatment with PRL during the observation period reduced the level of hyperglycemia from day 10 onwards (P<0.05) (Fig. 1). BC administration in combination with STZ did not significantly affect the elevation in blood glucose levels (Fig. 2). Morphologic examinations of the pancreas on day 21 of mice receiving STZ injections plus saline revealed a marked insulitis and structural changes of the islets (Table 1). This appeared to be modulated to some extent in the PRL treated animals (Table 1). The degree of insulitis seemed unaffected by BC (Table 1). PRL (Fig. 1) or BC (data not shown) administration alone did not change the serum glucose concentration or affect the pancreatic islet morphology, compared with the saline-treated group of animals (Table 1).

Effects of PRL on STZ induced islet dysfunction in vitro

In this series of experiments, pancreatic islets were pre-exposed for 30 min at 5·6 mM glucose to different treatments. After the islets were exposed to 5·6 mM glucose for 30 min, the islets were washed and the different treatments were added to the islets for 30 min. After the incubation period, the islets were washed again and placed in fresh media for 24 h. The results showed that PRL significantly reduced the secretion of insulin by the islets, while BC did not have any effect on the secretion of insulin.

Table 1 Effects of PRL and BC on islet morphology. Pancreatic islet histology rank in male C57BL/KsJ mice treated with one daily i.p. injection of STZ (40 mg/kg body weight; 0·2 ml) or saline 9 g/l; 0.2 ml) for 5 days. In addition, the mice were also given a second i.p. injection of either PRL (4 mg/kg body weight; 0·2 ml), saline, BC (10 mg/kg body weight; 0·2 ml) or vehicle (ethanol (70%):saline (1:9) and tartaric acid (10 mg/kg body weight); 0·2 ml) 30 min after the first type of injections and maintained daily for 21 days. The histology was ranked according to four arbitrary classes: A, normal islet structure; B, mononuclear cell infiltration in the periphery of the islets; C, infiltration of mononuclear cells into a majority of islets i.e. insulitis; D, only a few islets left, often with altered histology, for example pyknotic cell nuclei

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1–5</th>
<th>Day 1–21</th>
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<tr>
<td>Saline</td>
<td>5</td>
<td>0</td>
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<tr>
<td>Saline + PRL</td>
<td>5</td>
<td>0</td>
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<tr>
<td>STZ + Saline</td>
<td>1</td>
<td>2</td>
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<tr>
<td>STZ + PRL (new set of experiments)</td>
<td>3</td>
<td>4</td>
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<tr>
<td>STZ + vehicle</td>
<td>0</td>
<td>1</td>
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<td>STZ + BC</td>
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concentrations of PRL and then incubated for 30 min in 1.8 mM STZ. After incubation, the islets were cultured overnight in RPMI 1640+10% FCS, whereupon glucose-stimulated insulin release of the islets was examined. The control islets increased their insulin secretion about 7-fold at 16.7 mM glucose, as compared with the secretion at 1.7 mM glucose (Figs 3 and 4). The islets incubated in the presence of the two different concentrations of PRL alone responded similarly to glucose compared with the controls. STZ caused a marked inhibition of glucose-stimulated insulin release, and this was not counteracted by PRL. The islet DNA and insulin contents were not changed by the brief incubation with PRL (Fig. 5) Exposure to 1.8 mM STZ caused about 30% reduction of the insulin content, but no change in the islet DNA content. There was a trend that the islet insulin content after STZ exposure was increased after addition of PRL, but this effect did not attain statistical significance (Fig. 5).

Discussion

The aim of the present study was to investigate if PRL can affect the development of type 1 diabetes in mice treated with multiple low doses of STZ. Due to previously reported immunologic actions of PRL (Berczi 1993, Walker et al. 1993, Neidhart 1998) we hypothesized that PRL might potentiate the development of autoimmune type 1 diabetes in this animal model. However, our results indicate that PRL has a beneficial effect on hyperglycemia and insulitis in the early phase of autoimmune diabetes. The reason for this effect is unclear, but it could be that PRL in itself enhances β-cell function during a period when the β-cell mass is being reduced. Other possibilities are that PRL decreases the β-cell toxic effects of STZ or that PRL counteracts the autoimmune mechanisms leading to β-cell destruction.

Our results show that in vitro exposure of mouse pancreatic islets to PRL (6 and 60 mg/l), could not prevent the inhibitory effects of STZ on glucose-stimulated insulin secretion. This argues against the view that the protective effect of PRL against multiple low dose STZ induced diabetes would be mediated by a direct effect of PRL on STZ action. It is therefore unlikely that the effect of PRL on hyperglycemia and insulitis observed in vivo may be attributed to direct molecular interactions between PRL and STZ, or an interference with cellular events leading to β-cell damage inflicted by STZ.

Previous data have shown that long-term exposure to PRL stimulates insulin secretion and proliferation of
β-cells in islets of murine and human origin (Brelje et al. 1993, Stout et al. 1997). The possible beneficial action of PRL in the present study could thus be attributed to PRL-induced stimulation of β-cell function. However, we currently did not observe that PRL stimulated insulin release in vitro after short-term incubation. Moreover, an accurate quantification of β-cell replication in vivo in an animal with insulitis would be difficult to perform, due to the presence of infiltrating immune cells within the islets. Thus, we cannot exclude that PRL could have exerted a positive influence on the β-cell mass during the development of low dose STZ diabetes.

It has been reported that hyperphagocytosis in rodents causes deficiencies in both humoral and cell-mediated immunity (Gala 1991, Reber 1993) and administration of PRL to such animals restores their immune function. PRL receptors have been identified on the cell membranes of white blood cells, and lymphocytes have been shown to secrete a PRL-like substance (Gala 1991, Reber 1993, Neidhart 1998). Either too high or too low levels of PRL may affect these receptors and due to feed-back regulation, immunosuppressive actions by PRL may result. It is likely that PRL acts as a signal, regulating the immune cells in the present experiments. Macrophages are present in the pancreatic islets very early after multiple STZ injections in mice and this is obligatory for the subsequent development of hyperglycemia (Kolb–Bachofen et al. 1988). PRL may inhibit IL-1 production from the macrophages (Berczi 1997) and thereby promote an altered immune response by shifting the balance from a cell-mediated to a humoral immune response, which has been proposed earlier to affect the outcome of the autoimmune process in type 1 diabetes (Rabinovitch 1993, 1994, Adorini et al. 1996).

In the literature, only a few experimental studies have focused on the possible relationship between PRL and the development of type 1 diabetes. First, in humans, some protective effects of BC, a dopaminergic agonist decreasing the secretion of PRL (Sinha et al. 1976), on β-cell function was observed in diabetic patients treated for several months (Atkison et al. 1990). Secondly, with regard to the experimental animal models of type 1 diabetes, divergent results exist. A protective effect of BC against hyperglycemia was observed in diabetes prone female non-obese diabetic (NOD) mice (Hawkins et al. 1994) but, in contrast, the drug appeared to accelerate the onset of diabetes in NOD males and significantly increased the rate of insulin in both sexes (Durant et al. 1995).

BC, as well as other D2-dopaminergic agonists, may also have various metabolic effects, for example they produce a marked hyperglycemia in rodents (Durant et al. 1995). It also inhibited insulin release from isolated mouse pancreatic islets in vitro, suggesting that insulin release may be controlled by D2-dopaminergic receptors on the β-cells (El-Denshary et al. 1982). We could not confirm this finding since no significant difference in serum glucose concentration was observed in mice receiving PRL or BC+s saline or vehicle compared with the correspondingly saline+s saline or vehicle treated group of animals. Our study did not show any clear effect of BC, which should antagonize the action of PRL, on the incidence of diabetes in mice treated with multiple low doses of STZ. The in vivo dose and administration route (i.p.) of BC was selected based on previous reports (Sinha et al. 1976, Durant et al. 1995). Of course, we can not exclude that these doses were inadequate to affect the immune system in the C57BL/KJmice, or that another administration route would give a different result. Another interesting finding was that we observed hair loss in some animals given BC. This is a well known side effect of this drug in humans and at least it shows that BC in this experiment was pharmacologically active.

In summary, results from our study and from others suggest that PRL may have a beneficial effect in autoimmune type 1 diabetes. It is likely that this reflects a combined effect of PRL on both the neuroendocrine, the immune system and perhaps also on the regulation of glucose metabolism.

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