Molecular and Cellular Endocrinology 383 (2014) 126–136

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

# Mitogen-activated protein kinases and protein phosphatase 5 mediate glucocorticoid-induced cytotoxicity in pancreatic islets and β-cells

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ARTICLE INFO

*Article history:*
Received 18 June 2013
Received in revised form 15 December 2013
Accepted 16 December 2013
Available online 20 December 2013

*Keywords:*
Glucocorticoids
Apoptosis
Pancreatic islet
JNK
p38 MAPK
Protein phosphatase 5

ABSTRACT

Glucocorticoid excess is associated with glucose intolerance and diabetes. In addition to inducing insulin resistance, glucocorticoids impair β-cell function and cause β-cell apoptosis. In this study we show that dexamethasone activates mitogen-activated protein kinases (MAPKs) signaling in MIN6 β-cells, as evident by enhanced phosphorylation of p38 MAPK and c-Jun *N*-terminal kinase (JNK). In contrast, the integrated stress response pathway was inhibited by dexamethasone. A p38 MAPK inhibitor attenuated dexamethasone-induced apoptosis in β-cells and isolated islets and decreased glucocorticoid receptor phosphorylation at S220. In contrast, a JNK inhibitor augmented DNA fragmentation and dexamethasone-induced formation of cleaved caspase 3. We also show that inhibition of protein phosphatase 5 (PP5) augments apoptosis in dexamethasone-exposed islets and β-cells, with a concomitant activation of p38 MAPK. In conclusion, our data provide evidence that in islets and β-cells, p38 MAPK and JNK phosphorylation work in concert with PP5 to regulate the cytotoxic effects exerted by glucocorticoids.


## 1. Introduction

Hyperglycemia and Diabetes mellitus are important causes of mortality and morbidity worldwide. The number of people with impaired glucose tolerance or manifest type 2 Diabetes mellitus (T2DM) is rising in all regions of the world. A systemic analysis of health examination surveys and epidemiological studies show that between 1980 and 2008 there were nearly 194 million new cases of diabetes (Danaei et al., 2011). Of these, 70% can be attributed to population growth and aging, but the cause for the remaining 30% are likely due to environmental changes.

Glucocorticoids (GCs) are steroid hormones produced by the adrenal cortex. These hormones promote processes that raise blood glucose levels (Wajchenberg et al., 1984) and due to these effects, patients exposed to elevated levels of GCs for longer periods of time suffer a heightened risk of glucose intolerance or even overt diabetes (Raul Ariza-Andraca et al., 1998; van Raalte et al., 2009; Vegiopoulos and Herzig, 2007). Endogenous overproduction of GCs by the adrenal cortex, as observed in patients with Cushing's syndrome, is associated with an increased 30–40% risk of developing diabetes (Biering et al., 2000). T2DM is also over-represented in patients with elevated GCs at levels that are subclinical for the diagnosis of Cushing's syndrome (Di Dalmazi et al., 2012). Furthermore, in a retrospective study of 1258 human volunteers, a positive correlation was found between long term cortisol levels and the prevalence of metabolic syndrome, which is a risk factor for T2DM (Stalder et al., 2013).

Supraphysiological concentrations of GCs are also evident during treatment with GC-based drugs, which have become a mainstay therapy for conditions of inflammation, autoimmune disease and cancer (McDonough et al., 2008; Schacke et al., 2002). Current knowledge indicates that new-onset diabetes after starting low-dose GC treatment seems rare, but progression of existing impaired glucose tolerance to overt diabetes is more

*Abbreviations:* ASK-1, apoptosis signal-regulating kinase 1; dexa, dexamethasone; eIF2α, eukaryotic translation initiation factor 2α; GC, glucocorticoid; GR, glucocorticoid receptor; JNK, c-Jun *N*-terminal kinase; MAPK, mitogen-activated protein kinase; PP5, serine/threonine protein phosphatase 5; ROS, reactive oxygen species; RU, RU486; SB, SB203580; SP, SP600125; T2DM, type 2 diabetes mellitus; UPR, unfolded protein response.

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http://dx.doi.org/10.1016/j.mce.2013.12.010

common (van der Goes et al., 2010). At higher dosages, especially during long-term treatment, increased risk for diabetes and inadequate glucose control among patients on GC treatment is a clinically well-known side effect of GC therapy, that has been found both in patients with asthma (Dendukuri et al., 2002; Suissa et al., 2010) and with rheumatic diseases (Origuchi et al., 2011).

The systemic metabolic actions of GCs include a general decrease in the uptake and utilization of glucose alongside with an increase in hepatic gluconeogenesis (McMahon et al., 1988). Importantly, GCs antagonize the anabolic effects of insulin and thus causes insulin resistance. In skeletal muscle, GCs interfere with insulin signaling causing an inhibition of GLUT-4 mediated glucose uptake as well as inhibition of glycogen synthesis (Ruzzin et al., 2005; Weinstein et al., 1998). GC-induced hepatic insulin resistance results in impaired suppression of hepatic glucose production by insulin (Andrews and Walker, 1999). In addition, GCs directly increase endogenous glucose production by induction of several genes involved in hepatic carbohydrate metabolism (Vegiopoulos and Herzig, 2007). These GC-mediated effects on muscle and hepatic tissue are augmented by GC-induced release of non-esterified fatty acids (NEFA) and adipokines from adipose tissue (Galic et al., 2010). Taken together, increased circulating levels of GCs heighten the need for insulin in order to maintain glucose homeostasis.

In response to alterations in insulin sensitivity pancreatic β-cells can adjust their insulin secretory capacity to meet the need for glucose homeostasis. Thus, experimental studies performed both in healthy human subjects and in rodent models show that steroid treatment results in hyperinsulinemia but maintained normal fasting blood glucose levels and glucose tolerance (Ahrén, 2008; Binnert et al., 2004; Hollingdal et al., 2002; Nicod et al., 2003; Schneiter and Tappy, 1998; Willi et al., 2002). Compensatory β-cell hypertrophy is the most plausible explanation for the increased secretory capacity (Rafacho et al., 2011). However, when people with any degree of susceptibility towards glucose intolerance are given GCs, β-cells fail to adapt, and in such individuals GC treatment may disrupt glucose homeostasis (Besse et al., 2005; Grill et al., 1990; Henriksen et al., 1997; Larsson and Ahren, 1999; Wajngot et al., 1992). Similarly, in diabetes prone Zucker rats (Ogawa et al., 1992; Ohneda et al., 1993) or in normal rats and mice treated with high dosages of GCs, glucose homeostasis is disrupted (Fransson et al., 2013; Rafacho et al., 2009). The establishment of any direct GC effects on pancreatic β-cells under *in vivo* conditions is difficult since systemic metabolic consequences of GC treatment (e.g. glucose, NEFA) interfere with direct GC-mediated effects. Although direct evidence for β-cell apoptosis during development of steroid-induced diabetes is lacking, GC-mediated cytotoxicity conditions is a striking feature upon GC exposure to isolated islets and insulinoma cell lines (Avram et al., 2008; Ranta et al., 2006; Reich et al., 2012). While these direct negative effects of GCs on β-cells are well established, the molecular mechanisms regulating GC action in β-cells remain largely unknown.

To elucidate pathways that can regulate GC action in β-cells, we have in the present study investigated the role of mitogen-activated protein kinases (MAPKs) in the regulation of GC-induced cytotoxicity. Our results show that SB203580, a p38 MAPK inhibitor, protected against dexamethasone-induced cell death in islet cells and in clonal insulin-producing cells, whereas SP600125, a c-Jun *N*-terminal kinase (JNK) inhibitor, augmented this death. Furthermore, we show that inhibition of the enzyme serine/threonine protein phosphatase 5 (PP5) also enhances death of dexamethasone-exposed β-cells.

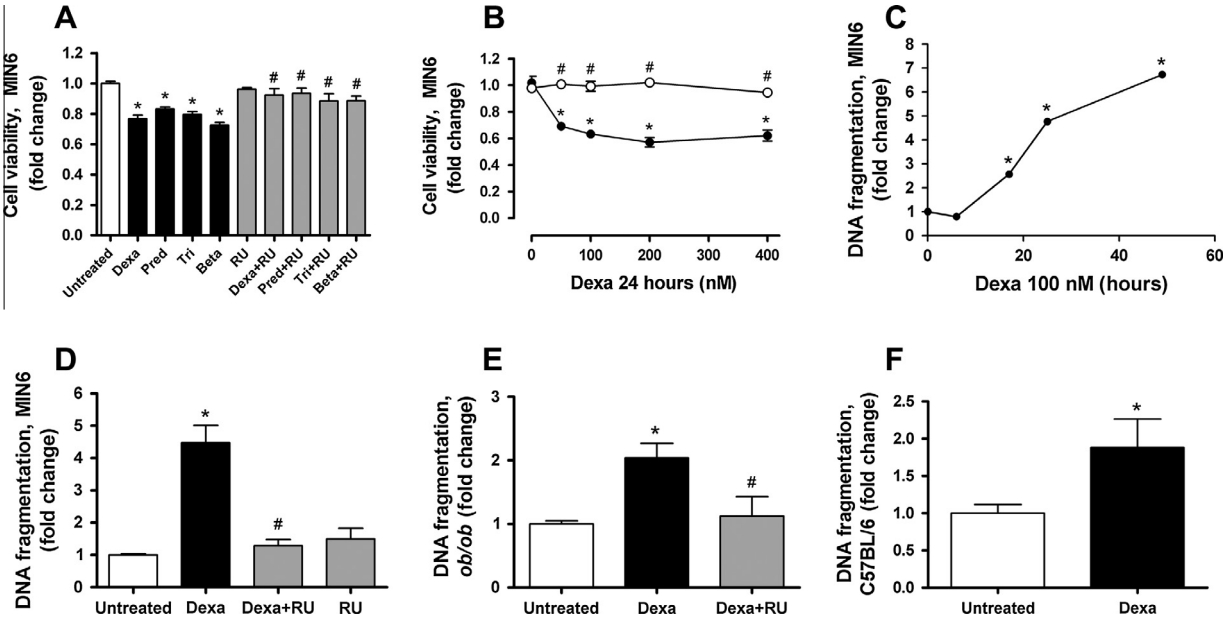


**Fig. 1.** GCs reduce cell viability in MIN6 cells and isolated islets of Langerhans. (A) MIN6 cells were cultured in the absence or presence of 100 nM of dexamethasone (Dexa), prednisolone (Pred), triamcinolone (Tri), or betamethasone (Beta) with or without 1 μM of RU486 (RU) for 24 h after which cells were lysed and lactate dehydrogenase activity was measured as indicator of cell viability ($n = 8$). (B) MIN6 cells were cultured at various concentrations of dexamethasone in the absence (closed circles) or presence (open circles) of 1 μM of RU486 for 24 h, after which cell viability was measured as above ($n = 8$). (C) MIN6 cells were cultured with 100 nM dexamethasone 0–48 h. Apoptosis was evaluated at different time points after exposure by measuring cytoplasmic levels of oligonucleosomes as an indicator of DNA fragmentation ($n = 6$). (D) MIN6 cells were cultured with 100 nM dexamethasone with or without 1 μM of RU486 for 24 h and apoptosis was evaluated as above ($n = 4$–15). Dexamethasone-induced apoptosis was also evaluated in islets of Langerhans isolated from obese *ob*/*ob* mice (E) or lean C57Bl/6J mice (F). Islets were exposed to dexamethasone and RU486 for 48 h after which apoptosis was detected as above ($n = 5$). Bars and circles represent mean ± SEM. A * denotes a significant ($p < 0.05$) difference from untreated cells and a # denotes a significant ($p < 0.05$) effect of RU486.