Morphofunctional Alterations in Endocrine Pancreas of Short- and Long-term Dexamethasone-treated Rats

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Key words
- beta-cell proliferation
- dexamethasone
- glucocorticoid
- insulin secretion
- insulin resistance
- short- and long-term treatment

Abstract

Long-term dexamethasone therapy may induce peripheral insulin resistance (IR), which in turn elicits increased beta-cell function and proliferation. However, whether such adaptive compensations occur during short-term treatment with dexamethasone is unclear. Here, we compared morphofunctional parameters in endocrine pancreas after short- and long-term dexamethasone administration. Groups of rats received daily i.p. injection of 1 mg/kg b.w. dexamethasone for 1 (DEX-1), 3 (DEX-3), or 5 consecutive days (DEX-5), whilst control rats were saline-treated (CTL). Despite the absence of apparent IR in DEX-1 rats, this group exhibited increased circulating insulin levels and glucose-stimulated insulin secretion (GSIS), compared to the CTL group ($p<0.05$). Evident IR as well as marked hyperinsulinemia and GSIS, as judged by the static and dynamic insulin secretion values, were observed in DEX-3 and DEX-5 rats ($p<0.05$). GSIS in islets cultured with 1 µM dexamethasone was lower compared to the control ($p<0.05$). Marked increases in beta-cell proliferation were observed in DEX-3 and DEX-5 rats, compared to CTL and DEX-1 rats ($p<0.05$). The alterations observed in DEX-3 rats were more pronounced in DEX-5 rats, which also exhibited a higher content of islet Cdk4 and Cd2 proteins, compared to the CTL group ($p<0.05$). We conclude that short-term dexamethasone treatment (DEX-1) induces an increase in beta-cell function that does not require the presence of discernible IR. As the treatment continues, the IR develops rapidly, and increased insulin secretion as well as beta-cell hyperplasia is demanded for the appropriate maintenance of glucose homeostasis.

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Introduction

Clinical use of glucocorticoids is based on their ability to reduce inflammatory processes and immune activation. Despite these desired effects, glucocorticoid therapies may also result in various undesirable effects [1]. Peripheral insulin resistance (IR) is one of these adverse effects and may be observed when dexamethasone is administered in excess. IR results from a direct glucocorticoid impairment of insulin action both in hepatic and extrahepatic tissues [2]. As pancreatic islet function is reciprocally related to peripheral insulin sensitivity, the amount of circulating insulin is adaptively increased during IR [3]. To account for this enhanced metabolic demand of insulin, adaptations in endocrine pancreas generally occur including augmentation in beta-cell function and proliferation [4–7]. Knowledge concerning these functional and structural adaptive compensations has derived from long-term dexamethasone experimental models. However, whether these islet compensations occur before, concomitant with, or after the development of dexamethasone-induced IR in rodent models is still a matter for debate.

Thus, the aim of this study was to characterize the development of IR, as well as the functional and structural alterations in the pancreatic islets during 3 distinct periods of dexamethasone treatment; after 1 (short-term), 3, or 5 consecutive days (long-terms) of daily high dose dexamethasone administration.

Materials and Methods

Materials

Dexamethasone phosphate (Decadron®) was purchased from Aché (Campinas, SP, Brazil). Human recombinant insulin (Biohulin® N) was from Biobrás (Montes Claros, MG, Brazil). The

reagents used in the insulin secretion protocols, radioimmunoassay (RIA), primary islet culture, histology and immunochemistry were from Mallinckrodt Baker, Inc. (Paris, Kentucky, France), Merck (Darmstadt, Germany), and Sigma (St. Louis, MO, USA). The ^{125}I-labeled insulin (human recombinant) for RIA assay was purchased from PerkinElmer (Waltham, MA, USA). SDS-PAGE and immunoblotting was performed using Bio-Rad systems (Hercules, CA, USA) and all chemicals used were from Bio-Rad and from Sigma.

Animals and dexamethasone treatment

Experiments were performed on groups of male Wistar rats (3-months old). The rats were obtained from the University of Campinas Animal Breeding Center and were kept at 24 °C on a 12-h light/dark cycle. Rats had access to food and water ad libitum. Experiments with animals were approved by the institutional São Paulo State University Committee for Ethics in Animal Experimentation and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23 revised 1996). Rats were distributed as follows: rats that received daily i.p. injection of 1 mg/kg b.w. dexamethasone for 1 (DEX-1) (short-term), 3 (DEX-3) or 5 (DEX-5) consecutive days (long-terms), whereas the control (CTL) group received saline (NaCl 0.9%) for 5 consecutive days, between 7:30–8:30 h. Daily injection were adjusted in a manner that all groups of rats: CTL, DEX-1, DEX-3, and DEX-5 were sacrificed on the same day (the day after the last saline or dexamethasone administration).

Metabolic and hormonal measurements and peripheral sensitivity tests

Blood was collected from the tail tip of fasted (12–14 h) or fed rats and blood glucose levels were measured with a glucometer ("one touch" – Johnson & Johnson). Immediately afterwards, animals were sacrificed (exposure to CO_2 followed by decapitation) and the trunk blood was collected. The serum, obtained by centrifugation, was used to measure fasted or fed insulin, triglycerides, and total protein, and albumin levels according to Rafacho et al. [8]. Determination of hepatic glycogen was performed, as previously described [9]. Intraperitoneal insulin (ipITT) and glucose tolerance test (ipGTT) were performed in separate groups of rats, according to a detailed previous description [8].

Islet isolation, primary islet culture, islet insulin content, and static and dynamic secretion protocols

Islets were isolated by collagenase digestion of the pancreas. For primary islet culture, islets were isolated from normal adult Wistar rats and cultured for 18 h in RPMI-1640 medium supplemented with 100 IU of penicillin/ml, 100 µg of streptomycin/ml and 250 ng/ml amphotericin at 37 °C in a 5% CO_2/air atmosphere under the following conditions: 1) 11.1 mM glucose + 10% fetal bovine serum (FBS) without or 2) with 1.0 µM dexamethasone and 3) 5.6 mM glucose + 0% FBS without or 4) with 200 pM insulin (a concentration known to induce primary beta-cell proliferation in culture [10]). Islets from primary culture were used for static insulin secretion protocols, whilst freshly isolated islets from CTL and all dexamethasone-treated groups were used for determination of islet insulin content and insulin secretion protocols (static and dynamic), as described previously in detail [8].

Quantitative approaches in endocrine pancreas

To study the morphometric parameters of endocrine pancreas, 5 pancreases from each group were excised and processed according to a previous description [11].

Beta-cell proliferation and death

The beta-cell proliferation was estimated by the percentage of PCNA-positive cells from the total of insulin-positive cells, as described previously in detail [11]. Detection of DNA fragmentation in situ was visualized with the use of the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon International, Norcross, GA, USA). The beta-cell death was estimated by the percentage of TUNEL-positive cells from the total of insulin-positive cells, as described previously in detail [6].

Protein extraction and immunoblotting

Protein extraction and immunoblotting were carried out as previously reported in detail [6, 11].

Statistical analysis

Results are expressed as the means ± SEM of the indicated number (n) of experiments. Analysis of variance (one way – ANOVA), for unpaired groups, followed by Tukey post test was utilized for multiple comparisons of parametric data. The significance level adopted was $p < 0.05$.

Results

Characteristics of the rats

On the day of experiments, decreased body weight was observed in DEX-3 and DEX-5, compared to CTL and DEX-1 rats (**Table 1**; n = 10; p < 0. 05). The adrenal mass (bilateral glands) was reduced in all dexamethasone-treated groups (**Table 1**; n = 10 glands from 5 rats; p < 0.01). The fasting hepatic glycogen content values were 2.2- and 2.0-fold higher in DEX-3 and DEX-5, compared to CTL rats (**Table 1**; n = 10; p < 0.01). Blood glucose and serum parameters at fasting and fed conditions are shown in **Table 1**. Glycemic values were similar among the 4 groups at fasting state. Fed blood glucose tended to be higher in DEX-5 vs. CTL rats, but it was not significant (n = 10). The serum insulin values increased in a time-dependent manner in both nutritional states, being higher in all dexamethasone-treated groups than in CTL ones (**Table 2**; n = 10, p < 0.05). The levels of serum triglyceride were significantly enhanced in DEX-3 and DEX-5 rats under fasting condition, but only in DEX-5 rats in fed condition, compared to CTL group (n = 10, p < 0.05). Total serum protein and albumin concentrations were higher in DEX-3 and DEX-5 vs. CTL rats under fasting and fed conditions (**Table 2**; n = 10, p < 0.01).

Table 1 Body weight, adrenal mass, and hepatic glycogen in control (CTL) and 1-(DEX-1), 3-(DEX-3), and 5-day dexamethasone-treated rats (DEX-5)

	Body weight (g)	Adrenal mass (mg/ 100 g b.w.)	Hepatic glycogen (mg/100 mg b.w.)
CTL	362 ± 6	18.6 ± 0.5	2.1 ± 0.3
DEX-1	357 ± 10	15.6 ± 0.6[a]	2.7 ± 0.3
DEX-3	323 ± 9[a,b]	13.2 ± 0.7[a,b]	4.5 ± 0.2[a,b]
DEX-5	314 ± 8[a,b]	12.7 ± 0.5[a,b]	4.1 ± 0.3[a,b]

Values are means ± SEM; n = 10

[a] Significantly different vs. CTL and [b] vs. DEX-1

p < 0.05 for 1-way ANOVA using Tukey's post test

	CTL	DEX-1	DEX-3	DEX-5
Fasting				
Glucose (mg/dl)	90.6 ± 2.3	100.7 ± 2.3	98.5 ± 4.3	99.2 ± 3.6
Insulin (ng/ml)	2.4 ± 0.3	6.4 ± 0.5[a]	9.3 ± 1.3[a]	14.3 ± 2.1[a,b]
TG (mg/dl)	106.6 ± 14.7	126.0 ± 15.2	216.4 ± 24.3[a]	343.6 ± 38.1[a,b,c]
T-protein (g/dl)	7.1 ± 0.1	7.8 ± 0.2[a]	8.1 ± 0.1[a]	7.9 ± 0.1[a]
Albumin (g/dl)	3.2 ± 0.1	3.2 ± 0.1	4.3 ± 0.1[a,b]	4.6 ± 0.1[a,b]
Fed				
Glucose (mg/dl)	109.0 ± 2.0	109.2 ± 3.9	114.2 ± 4.7	137.2 ± 20.7
Insulin (ng/ml)	2.6 ± 0.4	5.9 ± 0.7[a]	11.5 ± 1.5[a]	15.7 ± 2.6[a,b]
TG (mg/dl)	189.0 ± 14.2	147.4 ± 18.6	187.9 ± 26.5	289.2 ± 26.2[a,b,c]
T-protein (g/dl)	6.1 ± 0.2	6.7 ± 0.1	7.0 ± 0.1[a]	7.2 ± 0.1[a]
Albumin (g/dl)	2.6 ± 0.1	3.1 ± 0.1[a]	3.5 ± 0.1[a,b]	3.8 ± 0.1[a,b]

Values are means ± SEM; n = 10

[a] Significantly different vs. CTL, [b] vs. DEX-1, and [c] vs. DEX-3

$p < 0.05$ for 1-way ANOVA using Tukey's post test

TG: triglycerides; T-protein: total protein

Table 2 Blood glucose and serum parameters in control (CTL) and 1-(DEX-1), 3-(DEX-3), and 5-day dexamethasone-treated rats (DEX-5)

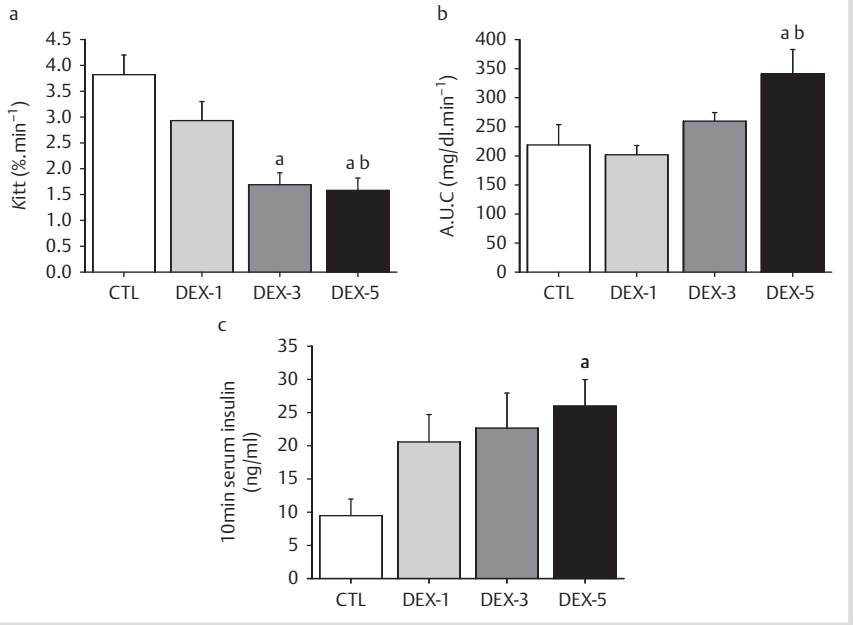

Fig. 1 Peripheral insulin and glucose sensitivity. **a**: The intraperitoneal insulin tolerance test in control (CTL), and 1-(DEX-1), 3-(DEX-3) and 5-day dexamethasone-treated rats. Note the decreased constant rate for glucose disappearance values (*K*itt) in DEX-3 and DEX-5 rats. Data are means ± SEM; n = 7. [a] Significantly different vs. CTL and [b] significantly different vs. DEX-1. $p < 0.05$ for 1-way ANOVA using Tukey's post test. **b**: The intraperitoneal glucose tolerance test in control (CTL), and 1-(DEX-1), 3-(DEX-3) and 5-day dexamethasone-treated rats. Note the augmented area-under-glucose-curve (AUC) values in DEX-5 rats. Data are means ± SEM; n = 7. [a] Significantly different vs. CTL and [b] significantly different vs. DEX-1. $p < 0.05$ for 1-way ANOVA using Tukey's post test. **c**: Insulin secretion in vivo after 10 min glucose load during ipGTT experiments. Data are means ± SEM; n = 5. [a] Significantly different vs. CTL. $p < 0.05$ for 1-way ANOVA using Tukey's post test.

Insulin sensitivity and glucose tolerance

The constant rate for glucose disappearance values (*K*itt), obtained through ipITT experiments, indicated a tendency towards a reduction in insulin sensitivity in DEX-1 (not significant) and a marked IR in DEX-3 and DEX-5, compared to CTL rats (**Fig. 1a**; $p < 0.001$). The *K*itt values were 3.8 ± 0.4, 2.9 ± 0.4, 1.7 ± 0.2, and 1.6 ± 0.2 %·min^{-1} for CTL, DEX-1, DEX-3, and DEX-5 rats, respectively (n = 7). DEX-5 rats showed decreased glucose tolerance after 2 g/kg glucose load, compared to CTL rats (**Fig. 1b**; n = 7, $p < 0.05$). The area-under-glucose-curve (AUC) values were 218.8 ± 35.0, 201.8 ± 16.1, 259.7 ± 14.7, and 341.1 ± 41.9 mg/dl·min^{-1} for CTL, DEX-1, DEX-3, and DEX-5 rats, respectively. After 10 min of glucose administration, the insulin response was increased in all rat groups (**Fig. 1c**; compare with fasting serum insulin values in **Table 1**). This insulin response to intraperitoneal glucose load was significantly higher in DEX-5, compared to CTL rats (n = 5, $p < 0.05$). The insulin response tended to be higher also in DEX-1 and DEX-3, compared to CTL group, but it was not statistically significant.

Insulin secretion from isolated islets

Experiments with static incubation revealed an augmented insulin secretion in response to increasing glucose concentrations in islets from all dexamethasone-treated rats, except for 2.8 mM glucose in DEX-1, compared to CTL islets (**Fig. 2a**; n = 15 wells, $p < 0.01$). **Fig. 2** shows the dynamic insulin release in perifused islets from CTL and dexamethasone-treated rats under 2.8 and 11.1 mM glucose. As expected, the biphasic insulin secretion was observed in CTL islets (**Fig. 2b**). Insulin secretion in islets from DEX-1, DEX-3, and DEX-5 rats also exhibited a biphasic pattern with higher insulin values than CTL islets (**Fig. 2c–e**). The area-under-curve (AUC) between the first 10 min after the introduction of 11.1 mM glucose revealed a significant increase in insulin response in islets from all dexamethasone-treated rats, compared to the CTL response (n = 4, $p < 0.05$). The AUC values were 631 ± 198, 8 740 ± 2 081, 12 662 ± 2 007 and 12 638 ± 2 474 pg/20 islets·ml^{-1}·min^{-1} for CTL, DEX-1, DEX-3, and DEX-5 rats, respectively. The AUC values obtained during the second phase (from 30 to 56 min) were higher in all DEX islet groups, compared to CTL group (n = 4, $p < 0.05$; only for DEX-3 group). The insulin secretion returned to basal values with the re-introduction of 2.8 mM glucose in all islet groups.

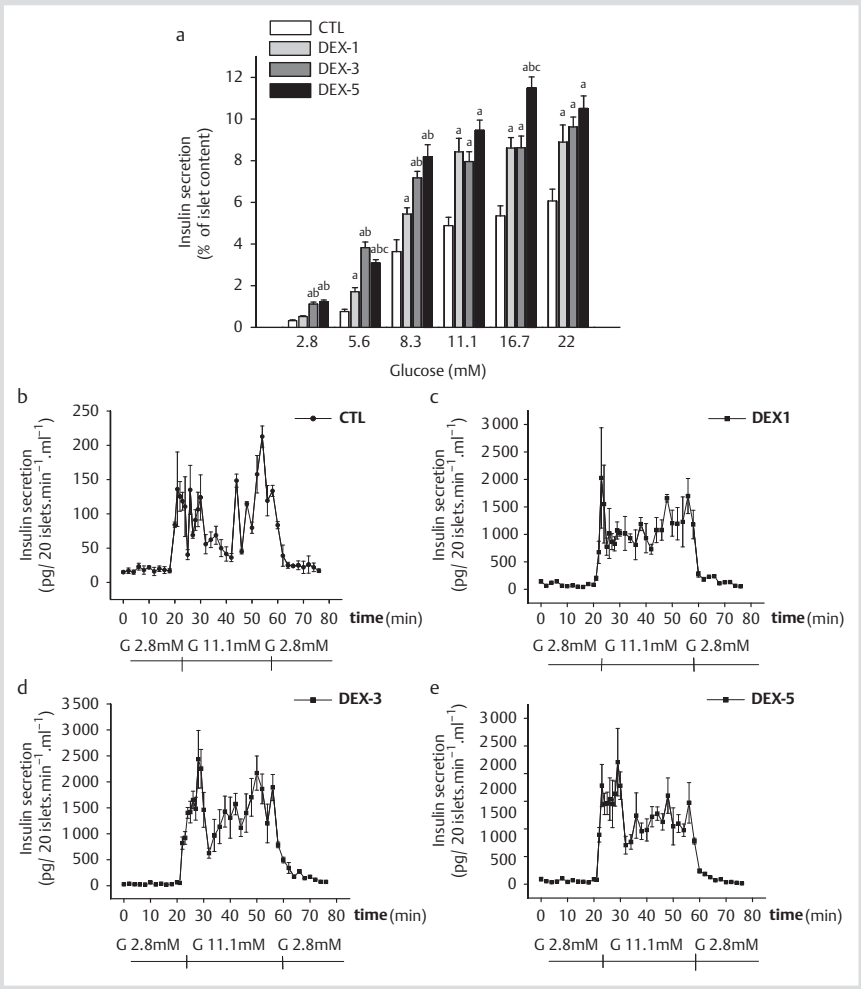

Fig. 2 Glucose-stimulated insulin secretion in freshly isolated islets. **a**: Cumulative static insulin secretion in islets isolated from control (CTL), and 1-(DEX-1), 3-(DEX-3) and 5-day dexamethasone-treated rats (DEX-5). Note the higher insulin response to crescent glucose stimuli in isolated islets from all dexamethasone-treated rats, in relation to CTL responses. Values are means ± SEM; n = 15 wells in 2 different experiments. [a] Significantly different vs. CTL, [b] vs. DEX-1 and [c] vs. DEX-3. $p < 0.05$ for 1-way ANOVA using Tukey's post test. **b–e**: Dynamic insulin secretion in islets isolated from CTL, DEX-1, DEX-3, and DEX-5 rats. Observe the biphasic insulin response in CTL (**b**) and the pronounced insulin response under 11.1 mM glucose stimulation in DEX-1 (**c**), DEX-3 (**d**), and DEX-5 (**e**) islets. Values are means ± SEM; n = 4 chambers in 2 different experiments.

Insulin secretion in primary islet culture

Isolated islets cultured for 18 h in the absence or presence of 1 µM dexamethasone was performed to test whether the direct effects of the glucocorticoid corroborates with the increased insulin response observed in vivo in DEX rats. As expected, glucose at stimulatory concentrations (11.1 or 22 mM) augmented insulin release in islets cultured in absence of dexamethasone, when compared to basal conditions (2.8 and 5.6 mM glucose) (**Fig. 3a**; n = 8 wells, $p < 0.05$). Islets cultured in presence of 1 µM dexamethasone did not exhibit such increase in insulin release under glucose stimuli (11.1 or 22 mM), compared to low glucose concentrations (2.8 and 5.6 mM) (n = 8 wells), being significantly reduced in relation to control values (**Fig. 3a**; $p < 0.05$). In another set of insulin secretion experiments, GSIS was performed in primary cultured islets (18 h) in the presence of 200 pM insulin to test whether this component could result in alteration of insulin response to glucose. According to **Fig. 3b**, islets cultured in 200 pM insulin medium had similar insulin response when stimulated with 11.1 or 22 mM glucose, compared to control islets (cultured with 5.6 mM glucose + 0% FBS) (n = 8 wells). The increase in GSIS was similar in both groups ($p < 0.05$).

Beta-cell proliferation and death

5-day dexamethasone administration induces increased pancreatic rat beta-cell proliferation [6, 11]. Herein, we confirmed the marked beta-cell proliferation, as judged by the large distribution of PCNA-positive nuclei in DEX-5, compared to CTL islets (**Fig. 4a, b** ; n = 40–50 islets, $p < 0.001$). DEX-3 rats also showed a higher frequency of beta-cell proliferation, compared to CTL ($p < 0.05$); however, this parameter was similar between DEX-1 and CTL rats (**Fig. 4a, b**). TUNEL analysis in pancreas sections did not reveal differences in beta-cell apoptosis between the 4 groups. The percentage of TUNEL insulin-positive cells were 0.16 ± 0.04, 0.14 ± 0.05, 0.13 ± 0.05, and 0.17 ± 0.06 for CTL, DEX-1, DEX-3, and DEX-5, respectively (n = 40–50 islets).

Cell-cycle protein levels

Irs-2 (rather than Irs-1), Akt, Cd1/2, and Cdk4 proteins are all associated with the control of beta-cell proliferation. Levels of Irs-1 and Irs-2 protein were similar among the 4 groups, although a tendency towards an increase in DEX-3 and DEX-5, compared to CTL islets was observed for both components (**Fig. 4c**; n = 6). Akt and Cd1 protein contents were also similar in islets of all groups. With regard to Cd2 protein, increases of 41% and 63% were observed in DEX-3 and DEX-5, respectively, vs. CTL islets (**Fig. 4c**; n = 6, $p < 0.05$ for DEX-5 only). Finally, levels of Cdk-4 protein were found to be higher (120%) in DEX-5 islets (n = 6, $p < 0.01$), and tended to be higher in DEX-1 and DEX-3, when compared to CTL islets (**Fig. 4c**, NS).

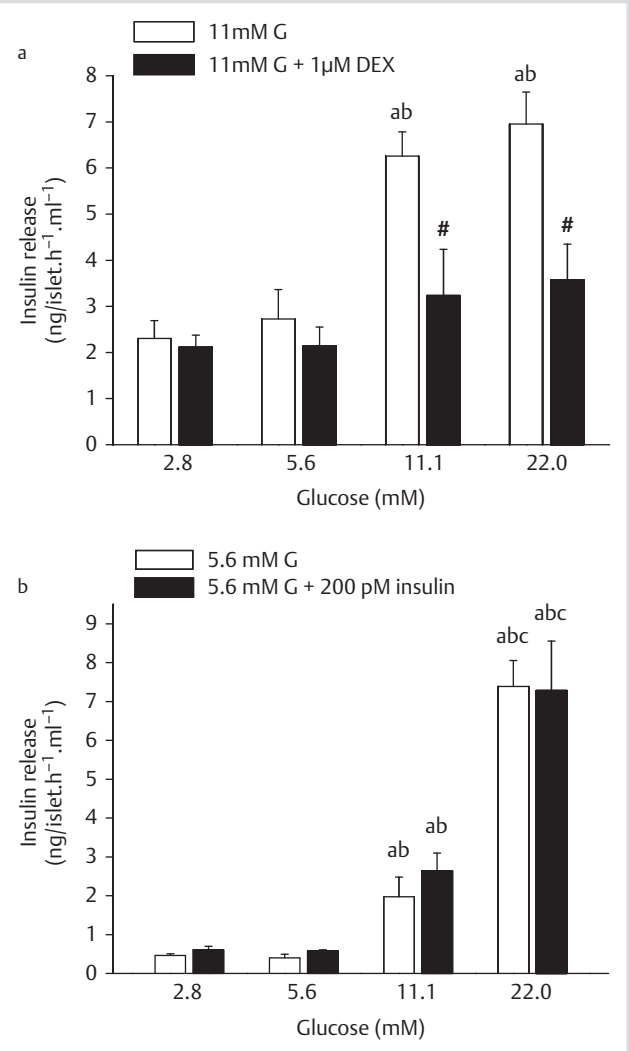

Fig. 3 Glucose-stimulated insulin secretion in primary islet culture. **a**: Cumulative static insulin secretion in islets cultured without or with 1 µM dexamethasone for 18 h. Observe the reduced insulin secretion in response to glucose stimuli in dexamethasone-cultured islets. Values are means ± SEM; n = 8 wells in 2 different experiments. [a] Significantly different vs. 2.8 mM and [b] vs. 5.6 mM glucose, and [#] Significantly different vs. control group (without dexamethasone). $p < 0.05$ for 1-way ANOVA using Tukey's post test. **b**: Cumulative static insulin secretion in islets cultured without or with 200 pM insulin for 18 h. Note similar glucose-stimulated insulin secretion in both islet groups. Values are means ± SEM; n = 8 wells in 2 different experiments. [a] Significantly different vs. 2.8 mM, [b] vs. 5.6 mM and [c] vs. 11.1 mM glucose. $p < 0.05$ for 1-way ANOVA using Tukey's post test.

Discussion and Conclusions

Long-term dexamethasone administration, at high doses, induces IR that is generally accompanied by several endocrine pancreas adaptive compensations. Among them, beta-cell hypersecretion [4, 7, 8, 12] and beta-cell hyperplasia [6, 11] guarantee the appropriate circulating insulin levels that keep the blood glucose at normal or near-normal physiological ranges. Whether these alterations may occur before, concomitant with or after the apparent development of IR is still a matter for debate. Thus, we investigated the effects of short-term (DEX-1), and long-term (DEX-3 and DEX-5) dexamethasone treatment on morphofunctional aspects of the endocrine pancreas in rats.

Dexamethasone-treated rat models are characterized by a reduction in body weight [8, 13], and increases in hepatic glycogen content [9, 14] and serum triglycerides [13] using a drug administration (1 mg/kg b.w.) varying from 5 to 11 consecutive days. Similar observations were found for our DEX-3 and DEX-5 rats, revealing that these characteristics are well reproducible and were present in DEX-5 rats from the third day of dexamethasone administration (DEX-3 rats). These metabolic disturbances were not observed in DEX-1 rats, suggesting that a single dose was not enough to induce them, even with a high dose of 1 mg/kg b.w.

With the exception of a marginal increase in fed blood glucose in DEX-5 rats, all groups exhibited normal glycemic range values, both in fasting and fed conditions. Dexamethasone-treated rats, including DEX-1 rats, showed higher serum insulin levels than those of the CTL group (**Table 2**). The marked hyperinsulinemia found in DEX-3 and DEX-5 rats is a well known compensatory adaptation that occurs in response to the striking IR observed in these groups. This compensatory response is demonstrated by previous studies in human and rats showing that this adaptation occurs from the second day of dexamethasone administration and continues thereafter, when the IR imposed by the glucocorticoid treatment is apparent [4, 5, 7, 15–17]. DEX-1 rats do not demonstrate a discernible IR (**Fig. 1a**), and the increase in serum insulin levels in this group cannot be attributed to a compensatory islet insulin response to peripheral insulin resistance. Although we did not detect such a response in our ipITT experiments, a previous study using the euglycemic-hyperinsulinemic clamp showed that a single dexamethasone dose (1 mg/kg b.w.) can induce a significant decrease in peripheral insulin sensitivity after 4 h of drug treatment [18]. Thus, a minimal effect of reduced insulin sensitivity in these animals may be present. However, Qi et al. [18] did not observe any increase in circulating insulin levels after 4 h. A possible explanation for the increased circulating insulin levels in DEX-1 rats may come from a central component. When administered directly to the central nervous system, dexamethasone increases the activity of the parasympathetic system resulting in augmented circulating insulin levels [19]. As previously shown, vagotomized rats treated with dexamethasone for 5 consecutive days at 1 mg/kg b.w., showed a lower level of hyperinsulinemia, but the same degree of insulin resistance, compared with control rats (without vagotomy) suggesting the participation of the cholinergic system in pancreatic islets during glucocorticoid treatment [20]. In an elegant study, Ahrén [21] demonstrated that the higher insulin response to arginine in insulin-resistant state, induced by dexamethasone treatment in healthy women, was reduced when dexamethasone treatment was conjugated with trimethaphan, a ganglia neurotransmission blocker. Thus, it is possible that our DEX-1 rats exhibit an early parasympathetic activation that results in such response. Given that DEX-1 rats have normal blood glucose and serum lipid concentrations, we cannot attribute a role for such circulating components on the process of insulin secretion in vivo during the period of 24 h.

Long-term dexamethasone treatment of adult rats with doses varying between 0.125 and 2.0 mg/kg efficiently augment the islet insulin response to several secretagogues, especially to glucose [4, 7, 8, 12, 16]. Increased GSIS in these animal models is mediated by augmented glucose responsiveness [4, 8, 16], mitochondrial function [7, 16], and intracellular calcium handling [7]. The protein kinases A and C [16, 22] and cholinergic pathway are also involved in this increased islet function under the

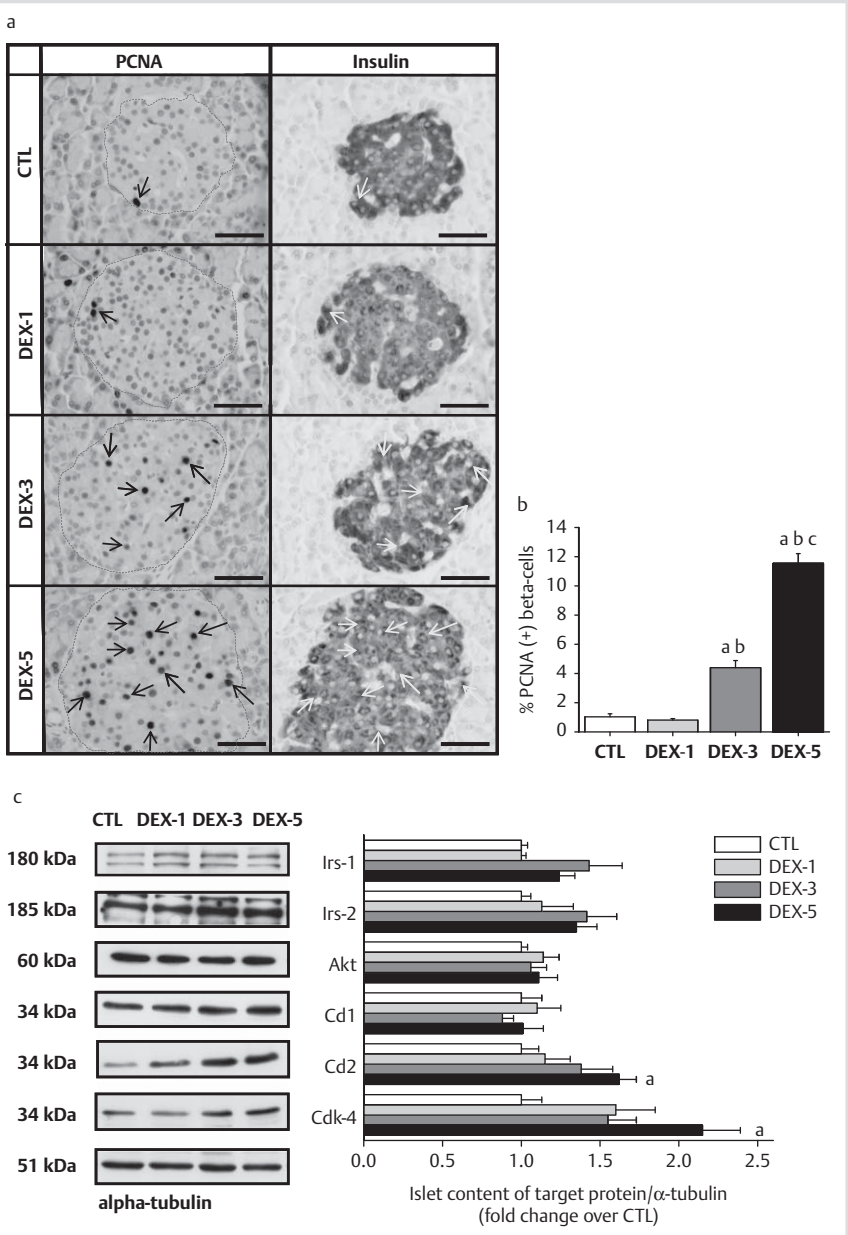

Fig. 4 Beta-cell proliferation and key cell-cycle protein levels. **a**: Representative images of pancreas sections stained for PCNA and insulin showing increased numbers of positive nuclei for PCNA (arrows) in beta cells from DEX-3 and DEX-5 rats, whilst this was rarely found in CTL and DEX-1 islets. **b**: Percentage of PCNA positive cell in beta-cells. Values are means ± SEM. n≅3 500 beta-cell nuclei. [a] Significantly different vs. CTL, [b] vs. DEX-1 and [c] vs. DEX-3. $p < 0.001$ for 1-way ANOVA using Tukey's posttest. Scale bars = 100 µm. **c**: Western blot experiments with islet lysates from control (CTL), and 1-(DEX-1), 3-(DEX-3) and 5-day dexamethasone-treated rats (DEX-5). DEX-5 rats showed significant increases in Cd2 and Cdk4 protein contents, compared to CTL ones. Below the panel, a representative control blot for alpha-tubulin is shown. Values are means ± SEM. The figures are representative of immunoblots performed at least five times on separate islet extracts. [a] Significantly different vs. CTL $p < 0.05$ for 1-way ANOVA using Tukey's post test.

glucocorticoid-induced IR [20]. Interestingly, GSIS from DEX-1 islets is significantly augmented when challenged by different glucose stimuli (**Fig. 2a, c**). Such insulin response may not be attributed to a direct effect of dexamethasone since primary cultured islets, in the presence of 1 µM dexamethasone, had decreased insulin response to stimulatory glucose concentrations (**Fig. 3a**). Furthermore, the role of elevated circulating insulin on the islet response to glucose seems to be not significant, at least for a short period, as judged by the similar GSIS in islets cultured with 200 pM insulin, compared to those cultured without insulin (**Fig. 3b**). Thus, additional studies merit investigation to explore the mechanisms underscoring beta-cell adaptation upon such a condition.

Conditions in which the demand by insulin is normally increased, such as obesity [23] and exogenous glucocorticoid administration [6,8], require an elevation in circulating insulin levels that is achieved by increased beta-cell function. Depending on the magnitude of the IR, augmentation in beta-cell mass participates in the compensatory adaptations. Increases in beta-cell mass, induced by long-term dexamethasone treatment, results from both beta-cell hyperplasia [6,11] and hypertrophy [6]. Beta-cell proliferation was markedly increased in DEX-5 rats (**Fig. 4a, b**) and is associated with an augmented cyclin d2 (Cd2) and cyclin-dependent kinase 4 (Cdk4) protein content in islets (**Fig. 4c**). Of note, we showed a 4.4-fold increase in beta-cell proliferation in DEX-3 rats, suggesting a high requirement for insulin from the third day of dexamethasone treatment to enable maintenance of glucose homeostasis. Since DEX-1 rats do not exhibit any alteration in beta-cell growth (proliferation and death), we excluded the possibility that dexamethasone exerts a possible direct effect on beta-cell proliferation in vivo. Rather, this seems to be a process that strictly depends on a permanent IR, suggesting a coupling between the peripheral tissues needed for insulin and pancreatic beta-cell function. It is important to emphasize that most morphofunctional alterations induced by the dexamethasone treatment in rats are reversible with the interruption of the treatment [22].

Therefore, we conclude that short-term dexamethasone treatment (a period of 24-h duration) induces an increase in beta-cell function, which does not require the presence of discernible IR. As the treatment continues, IR develops, and increased insulin secretion and beta-cell hyperplasia are observed in a time-dependent manner that accounts for the appropriate maintenance of glucose homeostasis.

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