

Investigation of the mechanisms contributing to the compensatory increase in insulin secretion during dexamethasone-induced insulin resistance in rhesus macaques

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Abstract

Dexamethasone has well-described effects to induce insulin resistance and increase insulin secretion. Herein, we examined potential contributors to the effect of dexamethasone to increase insulin secretion in rhesus macaques. Six male rhesus macaques received daily injections of either saline or dexamethasone (0.25 mg/kg i.m. for 7 days) in random order with 3 weeks between treatments. At the end of the treatment period, animals were fasted overnight and underwent a feeding study the next day, during which blood samples were taken before and for 60 min after a meal in order to assess islet hormone and incretin secretion. Dexamethasone induced marked increases in fasting plasma insulin, glucagon, leptin, and adiponectin concentrations ($P < 0.05$). Surprisingly, the glycemic response after meal ingestion was decreased twofold during dexamethasone treatment ($P < 0.05$). Dexamethasone-treated animals exhibited a significant increase in both insulin and glucose-dependent insulinotropic polypeptide (GIP) secretion during the feeding study ($P < 0.05$). However, glucagon-like peptide-1 secretion was significantly lower in dexamethasone-treated animals compared with controls ($P < 0.01$). Fasting and meal-stimulated pancreatic polypeptide concentrations (an index of the parasympathetic input to the islet) did not differ between saline and dexamethasone treatments. However, the proinsulin:insulin ratio was decreased throughout the feeding study with dexamethasone treatment suggesting an improvement of β -cell function ($P < 0.05$). In conclusion, the maintenance of euglycemia and reduction of postprandial glycemia with short-term dexamethasone treatment appears to be due to the marked elevations of fasting and meal-stimulated insulin secretion. Furthermore, increases in postprandial GIP secretion with dexamethasone treatment appear to contribute to the effect of dexamethasone treatment to increase insulin secretion.

Key Words

- ▶ insulin resistance
- ▶ Dexamethasone
- ▶ GIP
- ▶ rhesus macaque

Journal of Endocrinology
(2013) 216, 207–215

Introduction

Dexamethasone is a synthetic glucocorticoid that is commonly used in the treatment of numerous medical conditions due to its anti-inflammatory and immunosuppressive properties (Rosen & Miner 2005). However, chronic exposure to high levels of circulating exogenous and endogenous glucocorticoids is associated with several side effects, including insulin resistance (Vegiopoulos & Herzig 2007). For example, patients with enhanced endogenous glucocorticoid production (Cushing's syndrome) often exhibit hyperglycemia, hyperinsulinemia, and hepatic steatosis (Howlett *et al.* 1985).

Under normal physiological conditions, glucocorticoids function to protect against hypoglycemia during times of acute stress and/or reduced energy intake. Glucocorticoids increase circulating glucose concentrations through several mechanisms; specifically, they increase hepatic glucose production (McMahon *et al.* 1988), decrease peripheral glucose uptake (Weinstein *et al.* 1998, Sakoda *et al.* 2000), and promote the breakdown of muscle and fat to provide substrates for gluconeogenesis (Divertie *et al.* 1991, Hasselgren 1999).

While long-term glucocorticoid treatment is often associated with derangements of glucose homeostasis, short-term glucocorticoid treatment (i.e. <2 weeks exposure) generally does not result in marked glycemic alterations (e.g. diabetes). Specifically, glucose homeostasis has been proposed to be maintained during short-term glucocorticoid treatment by the induction of hyperinsulinemia (Karlsson *et al.* 2001, Nicod *et al.* 2003), which is supported by both clinical studies in humans and studies in rodents demonstrating marked increases in glucose-stimulated insulin secretion during short-term glucocorticoid treatment (Beard *et al.* 1984, Rafacho *et al.* 2010a). However, the mechanism(s) by which dexamethasone causes these marked elevations of insulin secretion are not well defined.

Longer term studies in rodents suggest that the hyperinsulinemic effect of glucocorticoids is due to an enhancement of β -cell mass and function (Ogawa *et al.* 1992, Rafacho *et al.* 2010b). However, there are little data on the mechanisms responsible for the observed enhancement of insulin secretion in the short term. We hypothesized that dexamethasone may enhance postprandial insulin secretion by increasing meal-stimulated incretin hormone secretion and/or by increasing parasympathetic input to the pancreas. The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are produced

and secreted by gastrointestinal enteroendocrine cells in response to the ingestion of nutrients (Baggio & Drucker 2007). Specifically, GLP-1 is produced by L-cells, primarily located in the distal gastrointestinal tract, and GIP is produced by K-cells, mostly located in the duodenum and proximal jejunum. As incretins, these hormones potentiate glucose-stimulated insulin secretion. Furthermore, studies in rodents suggest that both these hormones promote increases in insulin synthesis and β -cell mass (Brubaker & Drucker 2004, Kim *et al.* 2005).

Furthermore, previous studies in rodents have suggested that the effect of glucocorticoids to increase insulin secretion is mediated, in part, by the parasympathetic nervous system (Angelini *et al.* 2010). Indeed, Fletcher & McKenzie (1988) demonstrated that administration of a parasympatholytic (atropine) to obese Zucker rats receiving corticosterone reduced the effect of corticosterone to enhance glucose-stimulated insulin secretion.

Thus, in order to investigate the role that incretin hormones and parasympathetic input to the pancreas play in the effect of short-term dexamethasone treatment to increase glucose-stimulated insulin secretion, we performed feeding studies in rhesus macaques after a 7-day course of dexamethasone treatment.

Materials and methods

Animals and catheter implantation

Six adult male rhesus macaques (*Macaca mulatta*) ranging 6–14 years of age (mean \pm S.E.M., 10.5 \pm 1.2 years) and 8.7–12.5 kg in body weight (mean \pm S.E.M., 10.7 \pm 0.6 kg) were used for the studies. A physical examination, complete blood count, and serum biochemistry panel were performed for each animal before animals were selected and placed on the study. The animals were acclimated to several hours of chair restraint before the study. At least 1 week before the study, an iliac or femoral artery was catheterized under ketamine/isoflurane anesthesia with a polyurethane catheter connected to a subcutaneous vascular access port (Access Technologies, Skokie, IL, USA), as described previously (Havel & Valverde 1996). Animals were housed in the American Association for Accreditation of Laboratory Animal Care accredited facilities at the California National Primate Research Center in accordance with standards established by the U.S. Animal Welfare Act and the Institute of Laboratory Animal Resources. The experimental protocols were approved by the Institutional Animal Care and Use

Committee at the University of California, Davis, and the California National Primate Research Center and were conducted in accordance with guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals.

Feeding protocol

Each animal was studied twice in random order: 1) during saline treatment and 2) during dexamethasone treatment (0.25 mg/kg per day i.m. for 7 days). The studies were performed at least 21 days apart. Animals received treatment for 7 days and then were fasted overnight and underwent a feeding study the following day as described previously (D'Alessio *et al.* 2001). Animals were placed in restraint chairs at least 1 h before initiation of the feeding study. Three baseline blood samples (3 ml each) were drawn from the arterial port at -15, -10, and 0 min. Animals were then fed food at 0 min and blood samples were drawn at 2 and 5 min after feeding food. Animals were then fed a meal of one banana, half an apple, and three monkey chow biscuits (Purina 5047, Ralston Purina Co., St Louis, MO, USA). Blood samples were then collected at 2, 5, 10, 15, 30, 45, and 60 min after the animals began to eat. In the subsequent experiment, the animals were fed the same amount and type of food that was consumed by that animal during the initial experiment. Therefore, the amount of energy consumed and the macronutrient composition of the meal did not differ between experiments. The meals contained an average of 38.6 ± 5.3 g carbohydrate, 5.0 ± 2.0 g protein, and 1.7 ± 0.4 g fat. The approximate macronutrient composition of the meal was 80, 13, and 7% carbohydrate, protein, and fat respectively.

Plasma assays

Blood samples collected for measurement of plasma glucose concentrations were drawn and placed in heparin-treated tubes. Blood samples for measurement of circulating insulin, pancreatic polypeptide, total GIP, and total GLP-1 concentrations were placed in tubes containing ethylenediamine tetraacetate and aprotinin (Sigma). Plasma glucose concentrations were measured by the glucose oxidase method with a glucose analyzer (Beckman Coulter, Inc., Fullerton, CA, USA). Plasma insulin concentrations were measured by RIA (Linco, St Louis, MO, USA). Plasma pancreatic polypeptide, GLP-1, and GIP concentrations were measured by RIA, as described previously (Gingerich *et al.* 1978, Morgan *et al.* 1978, Orskov & Holst 1987).

Statistical analysis

Data are presented as mean \pm S.E.M. Statistical analyses were performed using GraphPad Prism 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Fasting plasma data were analyzed by one-factor ANOVA with Bonferroni's multiple comparison posttest analyses. Data from the feeding studies were analyzed by Student's *t*-test of the area under the curve (AUC). Differences were considered significant at $P < 0.05$.

Results

Fasting metabolites

Fasting plasma cortisol concentrations dropped to below the detection limit of the cortisol assay throughout the 7-day course of dexamethasone treatment, due to negative feedback by the exogenous glucocorticoid on the hypothalamic-pituitary-adrenal axis (Fig. 1A; $P < 0.001$). Dexamethasone treatment resulted in marked fasting hyperinsulinemia (control, 102 ± 24 pmol/l; dexamethasone, 906 ± 228 pmol/l; $P < 0.05$), reflecting the development of insulin resistance. Fasting plasma proinsulin concentrations were also markedly increased during dexamethasone administration (Fig. 1B; $P < 0.01$). However, fasting plasma glucose concentrations were unchanged during dexamethasone treatment (control, 4.0 ± 0.2 mmol/l; dexamethasone, 4.3 ± 0.2 mmol/l). Interestingly, fasting plasma glucagon concentrations increased in a step-wise manner during dexamethasone treatment (Fig. 1C; $P < 0.05$).

Changes of circulating leptin and adiponectin levels were also assessed. Similar to what has been reported in previous clinical studies in humans, dexamethasone treatment resulted in a significant elevation of fasting plasma leptin concentrations within 2 days of treatment (Dagogo-Jack *et al.* 1997), which persisted over the 7-day treatment period (Fig. 1D). Fasting plasma adiponectin concentrations were also significantly elevated with dexamethasone treatment (control, 355 ± 42 pmol/l; dexamethasone, 446 ± 36 pmol/l; $P < 0.05$).

Postprandial glycemic response

Feeding studies were performed in order to assess the effect of dexamethasone treatment on glucose homeostasis in response to a meal. As shown in Fig. 2A, basal fasting plasma glucose concentrations did not differ with saline or dexamethasone treatment. Plasma glucose concentrations did not change during the period in which animals were

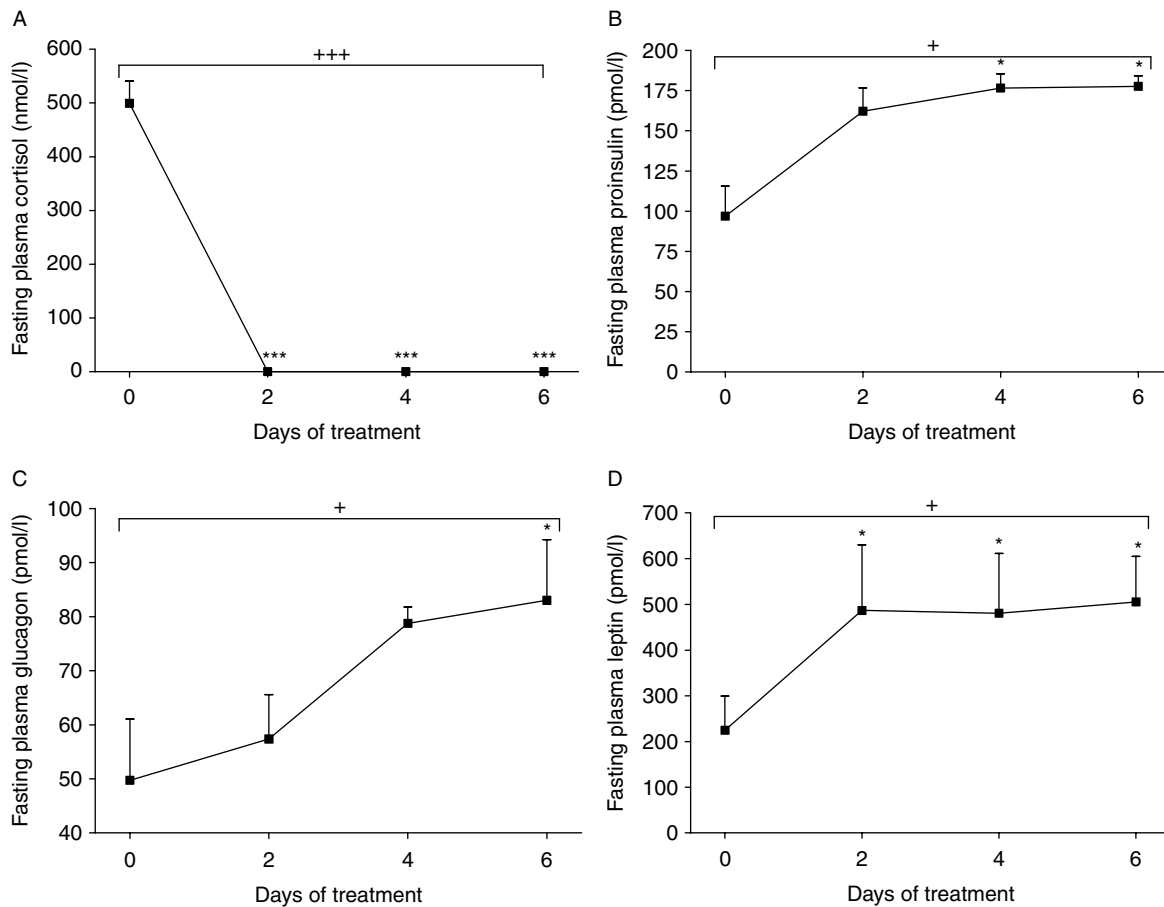


Figure 1

Fasting plasma hormones. Fasting plasma concentrations of cortisol (A), proinsulin (B), glucagon (C), and leptin (D). $+++P < 0.001$, $+P < 0.05$ by one-factor ANOVA, $***P < 0.001$, $*P < 0.05$ by Bonferroni's posttest compared with baseline.

fed food, under either treatment condition. However, under control conditions, plasma glucose concentrations rose significantly above baseline concentrations starting at 10 min after the start of the meal and remained significantly elevated for the 1-h duration of the feeding study. By contrast, with dexamethasone treatment, animals did not exhibit significant increases in circulating glucose levels until 30 min after the initiation of the meal. Furthermore, the glucose AUC during the meal was reduced by ~50% with dexamethasone treatment (control, 174 ± 33 mmol/l \times 60 min, dexamethasone, 79 ± 28 mmol/l \times 60 min; $P < 0.05$).

As shown in Fig. 2B, insulin concentrations were markedly elevated with dexamethasone treatment compared with saline-treated controls at all time points during the feeding test. Moreover, insulin concentrations did not change from baseline levels under either treatment condition when animals were shown food. Under control conditions, circulating insulin

concentrations rose significantly above baseline starting at 15 min after initiation of the meal. However, when animals were treated with dexamethasone, insulin concentrations rose significantly above baseline starting at 20 min after initiation of the meal. Dexamethasone treatment resulted in approximately a fourfold increase in the insulin AUC during the meal compared with control conditions (control, $21\,148 \pm 5056$ pmol/l \times 60 min; dexamethasone, $91\,916 \pm 19\,316$ pmol/l \times 60 min; $P < 0.01$). Furthermore, the increase in insulin concentrations from baseline to peak values was approximately threefold higher with dexamethasone treatment ($P < 0.05$). Thus, 7 days of treatment with dexamethasone markedly increased both fasting plasma insulin and postprandial insulin responses.

Glucagon concentrations were approximately threefold higher at all time points with dexamethasone treatment compared with control (Fig. 2C; $P < 0.01$). Plasma glucagon concentrations decreased after ingestion

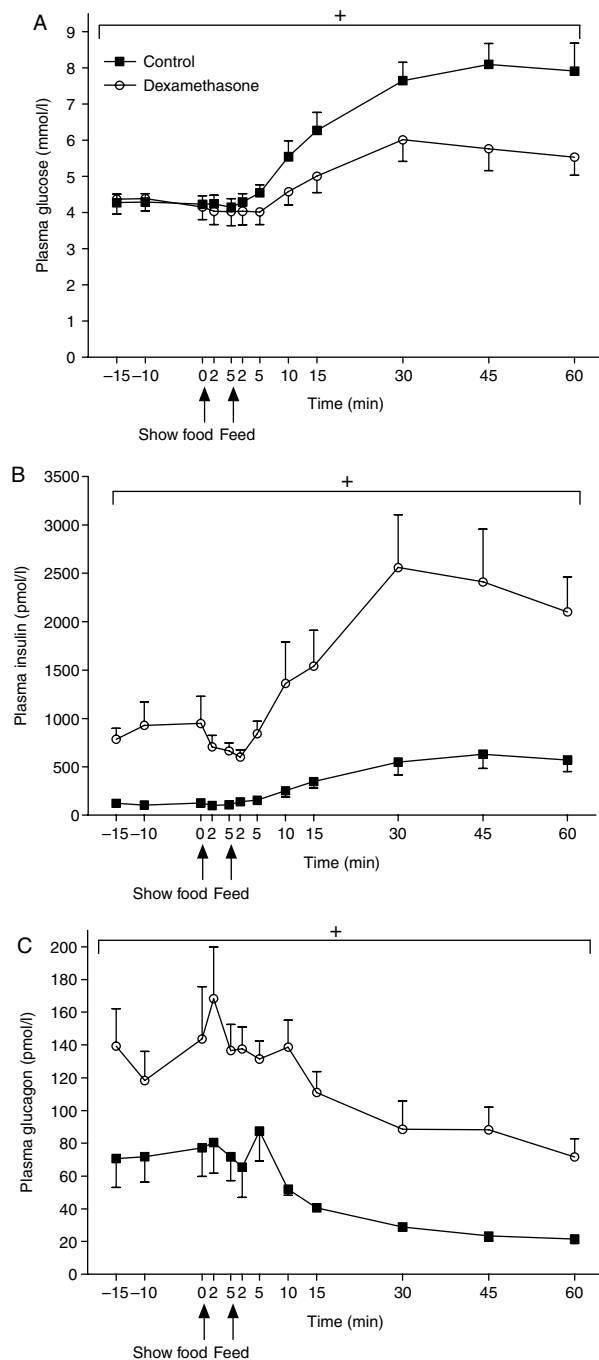


Figure 2 Postprandial glucose and pancreatic hormone responses. Plasma levels of glucose (A), insulin (B), and glucagon (C) during a feeding test. + $P < 0.05$ by Student's t -test of the AUC.

of the meal under both control and dexamethasone-treated conditions. The increase in fasting and postprandial plasma glucagon concentrations with dexamethasone treatment appears to be a compensatory response to the

marked increase in fasting plasma insulin concentrations and postprandial insulin secretion to prevent the development of hypoglycemia.

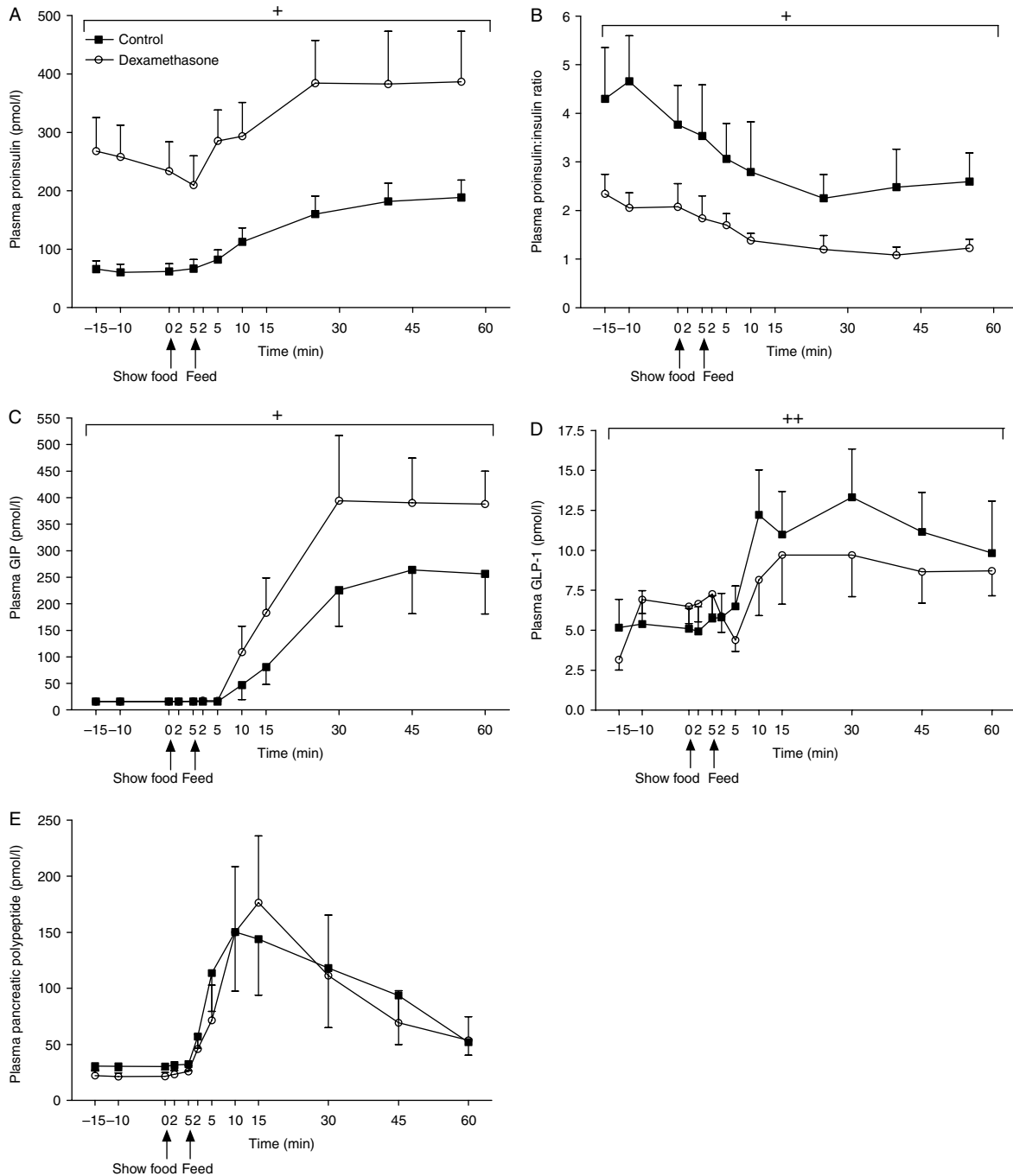
Postprandial proinsulin response

Proinsulin was measured before and during the feeding studies in order to assess β -cell function. Before the feeding test, fasting proinsulin concentrations were approximately threefold higher in dexamethasone-treated animals compared with controls ($P < 0.01$). Circulating proinsulin concentrations remained significantly higher throughout the feeding study when animals had been treated with dexamethasone (Fig. 3A; $P < 0.05$). The proinsulin to insulin ratio was calculated as an index of β -cell function in response to dexamethasone treatment. The proinsulin-to-insulin ratio was significantly lower in dexamethasone-treated animals compared with control conditions throughout the feeding study (Fig. 3B; $P < 0.05$). Thus, dexamethasone treatment appears to improve β -cell function.

Postprandial incretin response

In order to investigate the potential contribution of incretins to the enhancement of insulin secretion during short-term dexamethasone treatment, the GIP and GLP-1 responses during the feeding study were assessed. Fasting plasma GIP concentrations did not differ between treatments (Fig. 3C). Plasma GIP concentrations increased to levels greater than fasting at 15 min after initiation of the meal with both saline and dexamethasone treatment. Importantly, the AUC for GIP was approximately twofold greater when animals were receiving dexamethasone (control, 9519 ± 3041 pmol/l \times 60 min; dexamethasone, 16276 ± 4196 pmol/l \times 60 min; $P < 0.05$), suggesting that dexamethasone-mediated increases in postprandial GIP secretion may contribute to the increase in postprandial insulin secretion observed during dexamethasone treatment.

Like GIP, fasting plasma GLP-1 concentrations did not differ between the treatment groups (Fig. 3D). Plasma GLP-1 concentrations also increased to levels greater than baseline at 15 and 20 min after initiation of the meal during both saline and dexamethasone treatment respectively. In contrast to GIP, the GLP-1 AUC was significantly lower in dexamethasone-treated animals compared with controls (control, 359 ± 101 pmol/l \times 60 min; dexamethasone, 130 ± 118 pmol/l \times 60 min; $P < 0.01$). Therefore, GLP-1 does not appear to contribute to the effect of dexamethasone treatment to augment insulin secretion.

**Figure 3**

Postprandial proinsulin, proinsulin:insulin ratio, incretin (GIP and GLP-1), and pancreatic polypeptide responses. Plasma levels of proinsulin (A), the plasma proinsulin:insulin ratio (B), and plasma concentrations of GIP (C),

GLP-1 (D), and pancreatic polypeptide (E). ++ $P < 0.01$, + $P < 0.05$ by Student's *t*-test of the AUC.

Postprandial pancreatic polypeptide response

Pancreatic polypeptide was measured during the feeding studies in order to assess changes of parasympathetic input to the pancreas. Fasting plasma pancreatic polypeptide

concentrations did not differ between treatments (Fig. 2E). Furthermore, pancreatic polypeptide excursions during the feeding study did not differ between treatments. Therefore, changes of parasympathetic input to the

pancreas do not appear to contribute to the effects of dexamethasone administration to increase fasting insulin concentrations or postprandial insulin secretion.

Discussion

In this study, we investigated the role of incretin hormones and parasympathetic input to the pancreas in dexamethasone-induced increases in postprandial insulin secretion in rhesus macaques. Short-term dexamethasone administration resulted in marked increases in fasting and postprandial circulating insulin concentrations, allowing for the maintenance of normoglycemia despite marked insulin resistance. Dexamethasone treatment also resulted in a decrease in the proinsulin to insulin ratio, suggesting an improvement of β -cell function in the short term. Furthermore, dexamethasone treatment resulted in a twofold increase in postprandial GIP excursions, suggesting that increased GIP secretion is a plausible contributor to the increase in postprandial insulin secretion observed with administration of exogenous glucocorticoids.

Similar to previous studies in rodents and clinical studies in humans, short-term dexamethasone treatment markedly elevated fasting and postprandial insulin secretion (Beard *et al.* 1984, Fletcher & McKenzie 1988, Rafacho *et al.* 2010a). This marked increase in meal-stimulated insulin secretion with dexamethasone treatment likely contributed to the 50% lower glucose excursions during the feeding test. Of note, the effect of dexamethasone to increase insulin secretion *in vivo* is in contrast to several studies demonstrating that, *in vitro*, glucocorticoids such as dexamethasone decrease insulin secretion (Gremlich *et al.* 1997). Also, similar to previous reports, dexamethasone treatment significantly elevated fasting plasma leptin concentrations (Dagogo-Jack *et al.* 1997). Leptin has been strongly implicated in having an important role in the regulation of glucose homeostasis and thus may have contributed to the maintenance of normoglycemia in dexamethasone-treated rhesus macaques (Cummings *et al.* 2011, Morton & Schwartz 2011).

Previous studies in rodents have shown that glucocorticoid administration increases insulin secretion by enhancing islet secretory capacity (Rafacho *et al.* 2010a). Therefore, we measured circulating proinsulin concentrations as an index of β -cell function in the fasting and postprandial states. While fasting and meal-stimulated proinsulin concentrations were increased with dexamethasone treatment, the proinsulin-to-insulin ratio was significantly lower in dexamethasone-treated animals,

suggesting an improvement of β -cell function during short-term dexamethasone treatment. This enhancement of β -cell function may be due to an enhancement of stimulus secretion coupling, as previously suggested by two studies in rats (Rafacho *et al.* 2010a,b).

Notably, both fasting plasma glucagon concentrations and glucagon levels in the postprandial state were markedly increased with dexamethasone treatment. These increases were likely a compensatory response to protect against the development of hypoglycemia in the face of marked hyperinsulinemia. Interestingly, a previous human clinical study in humans by Wise *et al.* (1973) demonstrated that dexamethasone administration increased fasting plasma glucagon concentrations and glucagon secretion in response to a protein meal in both lean subjects and in subjects with increased endogenous glucocorticoid production (i.e. Cushing's syndrome).

Several mechanisms for glucocorticoid-induced increases in glucose-stimulated insulin secretion have been proposed in the literature, including increases in circulating incretin hormones (Koko *et al.* 2008) and enhanced parasympathetic input to the islet (Angelini *et al.* 2010). In this study, short-term administration of dexamethasone resulted in a twofold increase in meal-stimulated GIP excursions compared with control conditions, but modestly decreased postprandial GLP-1 excursions. These data are in agreement with previous work by Koko *et al.* (2008) demonstrating that 12 days of treatment with dexamethasone in Wistar rats increased intestinal K-cell numbers. Of note, fasting plasma GIP concentrations did not differ between treatments, whereas fasting plasma insulin concentrations were increased fourfold with dexamethasone treatment. This suggests that a mechanism other than GIP action on the β -cell is responsible for producing fasting hyperinsulinemia during administration of exogenous glucocorticoids. These results are also in partial agreement with a recent study in humans by Jensen *et al.* (2012) reporting that 5 days of treatment with dexamethasone results in increased GIP excursions during an oral glucose tolerance test. However, the observed decrease in GLP-1 excursions during the feeding study in rhesus macaques conflicts with this same study, which also demonstrated significant increases in GLP-1 excursions during the oral glucose tolerance test. In fact, GIP and GLP-1 are generally regulated in parallel; however, in this study, we report a divergent effect of dexamethasone on GIP and GLP-1 regulation. These differences may be due to species-related differences in the regulation of incretin hormone secretion. Furthermore, other causes of insulin resistance

have been shown to increase GLP-1 secretion. For example, a study performed in dogs reported increased GLP-1 secretion in insulin resistance induced by high-fat feeding (van Citters *et al.* 2002). This suggests that potential differential effects of insulin resistance on incretin secretion may be dependent on the underlying etiology of the insulin resistance. Nevertheless, the current results suggest that increased GIP, but not GLP-1, has a role in enhancing postprandial insulin secretion during short-term treatment with dexamethasone in rhesus macaques. However, a recent study in humans reported that the insulin secretory response to GIP and GLP-1 is impaired with experimental insulin resistance induced by a combination of increased energy intake and prednisolone administration for 12 days (Hansen *et al.* 2012). Additional studies will be necessary to determine the physiological role of increases in meal-stimulated GIP secretion in glucocorticoid-induced insulin resistance.

Several previous studies performed in rodents have suggested that increased parasympathetic outflow to the pancreas is an important contributor to the effect of glucocorticoids to increase insulin secretion (Fletcher & McKenzie 1988, Angelini *et al.* 2010). Pancreatic polypeptide is an islet hormone known to be closely regulated by parasympathetic input to the islet in response to feeding and during insulin-induced hypoglycemia in rhesus macaques and humans (Schwartz 1988, Havel & Valverde 1996, Havel & Ahren 1997, Ahren & Holst 2001, D'Alessio *et al.* 2001). In this study, we found that peripheral administration of dexamethasone to rhesus macaques does not alter fasting or meal-stimulated pancreatic polypeptide concentrations. Interestingly, a previous study by Sainsbury *et al.* (2001) demonstrated that central but not s.c. administration of dexamethasone to Wistar rats results in increased parasympathetic outflow to the pancreas and elevated insulin secretion. However, based on the measurements of pancreatic polypeptide concentrations in this study, it does not appear that increased parasympathetic input to the pancreas has a role in glucocorticoid-mediated increases in insulin secretion when dexamethasone is administered peripherally.

In conclusion, a 7-day course of dexamethasone treatment in rhesus macaques produces marked fasting and postprandial hyperinsulinemia in order to maintain normal fasting and postprandial glucose homeostasis. The increase in circulating insulin concentrations appears to overcompensate for the degree of insulin resistance; however, this is counteracted by increased glucagon concentrations in both the fasting and the postprandial states. The increase in postprandial insulin secretion

observed after dexamethasone administration may be mediated, at least in part, by increases in meal-stimulated GIP secretion. Increases in parasympathetic input to the pancreas do not appear to contribute to glucocorticoid-induced increases in insulin secretion, as pancreatic polypeptide responses were unchanged during dexamethasone treatment. Overall, these findings in non-human primates provide new information on the short-term compensatory alterations of pancreatic islet and incretin hormones occurring in response to short-term exogenous glucocorticoid administration.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by NIH grants DK-50129, DK-35747, and the Venture Research Program of the California Regional Primate Research Center (RR-00169), the Medical Research Council of Canada, the Canadian Diabetes Association, and the Alberta Heritage Foundation for Medical Research. Dr Havel's Laboratory also receives funding from NIH grants 1RC1DK087307-01, AT-002993, AT-003545, HL-075675, HL-091333, DK-095980, and HL-107256 and a Multicampus Award from the University of California, Office of the President.

Acknowledgements

The authors would like to acknowledge the expert technical assistance of Vanessa Bakula, Philip Allen, David Robb, Elaine Doray, Monica Lee, Sathiyabama Dayanandan, Ellen Laschansky, Kimber Stanhope, and Dr John Anderson. In addition, they wish to thank Drs Ronald L Gingerich and Michael Landt, and the Radioimmunoassay Core Laboratory of the Diabetes Research and Training Center at Washington University, St Louis, for performing the insulin, glucagon, and pancreatic polypeptide assays. They would also like to acknowledge Jenny Short and Sarah Davis for their help in coordinating the studies and Dr Andrew G Hendrickx for supporting the project.

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Received in final form 6 November 2012

Accepted 13 November 2012

Accepted Preprint published online 14 November 2012