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Effect of dexamethasone on insulin sensitivity, islet amyloid polypeptide and insulin secretion in humans

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Summary. The response of islet amyloid polypeptide and insulin and their molar ratios were investigated in eight healthy volunteers before and after treatment with dexamethasone by oral and frequently-sampled intravenous glucose tolerance tests. Following dexamethasone treatment the insulin sensitivity index decreased significantly from 6.5 ± 1.3 to 4.1 ± 1.0 $(\mu U \cdot ml^{-1}) \cdot min^{-1}$, $p < 0.05$. The area under the curve representing above-basal levels of insulin during oral glucose tolerance test increased significantly following dexamethasone treatment from 48132 ± 9736 to 82230 ± 14846 $pmol \cdot l^{-1} \cdot 3\ h^{-1}$, $p < 0.05$, the area under the curve of islet amyloid polypeptide increased from 1308 ± 183 to 2448 ± 501 $pmol \cdot l^{-1} \cdot 3\ h^{-1}$, $p < 0.05$. The overall insulin/islet amyloid polypeptide molar ratios calculated from the area under the curve during the 3-h period of the oral glucose tolerance test was not significantly different before and after dexamethasone treatment (42 ± 5 vs 40 ± 4). During the oral glucose tolerance test the insulin/islet amyloid polypeptide ratio increased significantly from baseline to 30 min ($p < 0.05$), then declined towards initial values before and after dexamethasone treatment. In conclusion, dexamethasone induced a significant decrease in insulin sensivity and a significant increase in insulin secretion during the oral glucose tolerance test. However, in contrast to previous animal experiments we did not find a change in the insulin/islet amyloid polypeptide ratio before and after dexamethasone treatment.

Key words: Islet amyloid polypeptide, insulin, dexamethasone, insulin resistance, Type 2 (non-insulin-dependent) diabetes mellitus.

Amyloidosis of the pancreatic islets is a common lesion in Type 2 (non-insulin-dependent) diabetes mellitus [1–3]. The discovery of islet amyloid polypeptide (IAPP) or amylin as the protein component of amyloid deposits [4, 5] has led to speculation about a possible role of IAPP in the pathogenesis of Type 2 diabetes. In particular, recent studies have focussed their interest not only on the ability of IAPP to form amyloid deposits but also on the effect of circulating IAPP on insulin sensitivity and insulin secretion, as a decrease in both is a prominent feature of Type 2 diabetes. IAPP is co-localized [6] and co-secreted with insulin from pancreatic beta cells [7–12]. Glucose-stimulated IAPP secretion is increased in obese subjects with normal and impaired glucose tolerance [11, 12]. In patients with Type 2 diabetes, IAPP secretion is decreased relative to that of insulin [11, 12].

The role of IAPP in the pathogenesis of Type 2 diabetes is still speculative. In vitro studies have demonstrated that infusion of IAPP inhibits glucose-stimulated insulin release by beta cells [13], and insulin-stimulated rates of glycogen synthesis and glucose uptake by skeletal muscle cells [14]. In animals IAPP infusion in pharmacological doses causes impaired insulin sensitivity in vivo [15, 16], which is a feature of Type 2 diabetes. However, other investigators have not found an effect of IAPP on insulin secretion [17]. In man, infusion of IAPP at a high concentration did not affect insulin secretion [18], which supports our finding that increased endogenous IAPP levels – as found in patients on haemodialysis – do not impair insulin secretion [19].

Co-secretion of IAPP with insulin has been shown by stimulation with glucose in humans [7–12] and with glucose, arginine and sulphonylureas in animals [20]. The effect of dexamethasone, an agent known to affect carbohydrate metabolism, on IAPP secretion has recently been examined in pancreatic tissues of rats [21, 22]. These studies demonstrate a greater increase of IAPP content compared to that of insulin, which may facilitate the formation of islet amyloid deposits.

The aim of this study was to examine the influence of dexamethasone treatment on insulin and IAPP secretion in healthy volunteers.

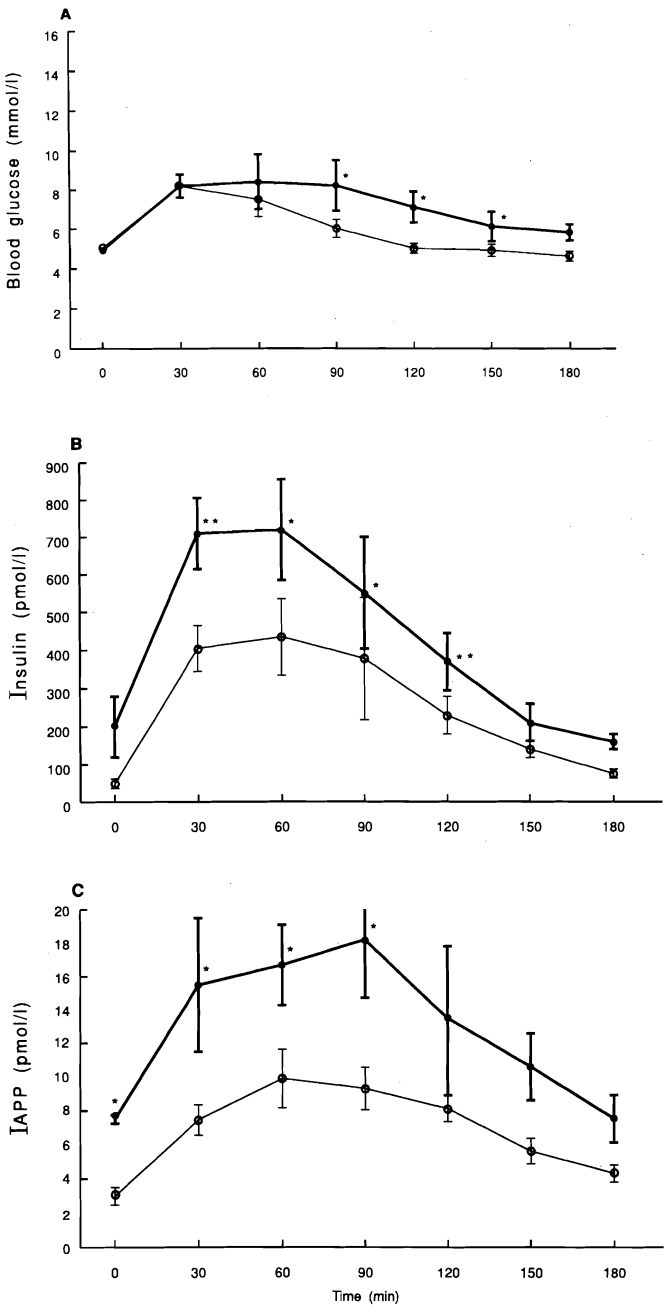

Fig. 1A–C. Serum levels of glucose (**A**), insulin (**B**) and islet amyloid polypeptide (IAPP) (**C**) during an oral glucose tolerance test before (○) and after (●) treatment with dexamethasone, * $p < 0.05$, ** $p < 0.01$

Subjects, materials and methods

We studied basal and glucose-stimulated plasma IAPP, insulin and glucose levels in eight healthy control subjects (five males, three females, mean age 26 ± 1 years, BMI 21.5 ± 0.8 kg/m^2). The study design was approved by the Ethical Committee of the University of Vienna and all the subjects gave their informed consent to take part. We performed an oral glucose tolerance test (OGTT), and a frequently-sampled intravenous glucose tolerance test (FSIGT) to measure insulin sensitivity assessed by the glucose disappearance minimal model of Pacini and Bergman [23]. The model accounts for the effect of insulin and glucose on glucose disappearance following the exogenous glucose injection. It provides the insulin sensitivity index (SI), defined as the ability of insulin to enhance glucose disappearance and to inhibit hepatic glucose production. The OGTT (75 g glucose) and the FSIGT (0.3 g glucose/kg body weight) were performed after an overnight fast on 2 different days. After the tests the volunteers received 4 mg dexamethasone orally every morning for 4 days. The OGTT and FSIGT were then each repeated on separate days.

Blood samples for measurement of glucose, insulin and IAPP (only for OGTT) were collected at 0, 30, 60, 90, 120, 150 and 180 min during the OGTT and at −1, 2, 3, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, 180, 210 and 240 min during the FSIGT.

Blood glucose was determined by an American Monitor-Parallel Analyser. Serum insulin was measured by commercial RIA (RIA-gnost Insulin; Behringwerke AG, Marburg, FRG).

IAPP was measured by a RIA developed in our laboratory as previously described [8, 11, 24]. The samples were assayed without knowledge of the status of the subjects in the protocol. First, IAPP is extracted from EDTA plasma (at least 5 ml) by Sep-Pak C18 cartridges (Waters/Millipore, Milford, Mass., USA) with methanol/tri-fluoroacetic acid (TFA)/water as the mobile phase (p. A. grade; Merck, Darmstadt, FRG). Then, IAPP is desorbed two times by 2 ml of methanol/TFA/water 90/0.5/9.5 by volume. The eluates are dried by a vacuum-concentrator (SVC 220 H; Savant Instruments, Farmingdale, NY, USA). For IAPP-RIA the dry plasma extracts are reconstituted in 350 µl of phosphate RIA-buffer and 100-µl aliquots in triplicate are incubated with 100 µl of rabbit-antihuman IAPP (Peninsula, Belmont, Calif., USA), simultaneously with 100-µl aliquots of the appropriate dilutions of amylin calibrator (range 4–260 fmol/ml) at +4 °C for 24 h. Then 100 µl of ^{125}I-IAPP-tracer (about 20000 cpm; Peninsula) is added for a further 24 h. Peptide bound to antibody is finally separated from unbound by precipitation with 0.5 ml antibody-immunoprecipitating reagent (sheep anti-rabbit; Sorin/Biomedica, Salluggia, Italy) for 30 min at room temperature, followed by addition of 1 ml of ice-chilled RIA-buffer and collection of the precipitate by centrifugation at +4 °C (5000 × *g*, 30 min). Supernatants are removed by suction and the radioactivity of the pellets is determined by gamma counting. IAPP-content of the samples is calculated by computer-aided processing of results from gamma-counting, using a logit-log-transformation of the calibrator curve.

We use dilutions of anti-IAPP, providing antibody binding of tracer at 0 dose of IAPP (B_0) in the range of 15% to 20% of total. Non-specific binding of tracer is less than 3% of total, the lowest detectable dose of IAPP clearly different from 0 is 0.4 fmol/tube; i. e., 0.3 pmol/l, if 5 ml of plasma are used (criterion B_0 − 3 SD). Calibrator doses equivalent to 80, 50, and 20% of tracer binding relative to 0 dose are 2.2, 6.2, and 18 fmol, respectively. Within- and between-run precision within this linear range of the RIA are 10 and 15%.

To test recovery of IAPP from plasma, three EDTA-plasma samples with low (< 1 pmol/l from patients with Type 1 (insulin-dependent) diabetes) and four samples with high (> 10 pmol/l) endogenous IAPP content were spiked by addition of 1,2 and 4 pmol/l synthetic IAPP. Recoveries ranged from 75% as the lowest and 90% as the highest extreme (85 ± 5%). IAPP values paralleled the calibrator curve, when 5-ml aliquots of plasma samples ($n = 5$) with endogenous IAPP content in the range of 6 to 15 pmol/l were diluted with 0.15 mol/l NaCl to plasma-NaCl ratios of 1 : 0.5, 1 : 1, 1 : 2, and 1 : 3.

Statistical analysis

All data are presented as means ± SEM. Statistical analysis was carried out by the paired Student's *t*-test. A *p*-value less than 0.05 was considered significant.

Results

Basal and stimulated levels of blood glucose, insulin, and IAPP during OGTT before and after treatment with dexamethasone are shown in Figure 1.

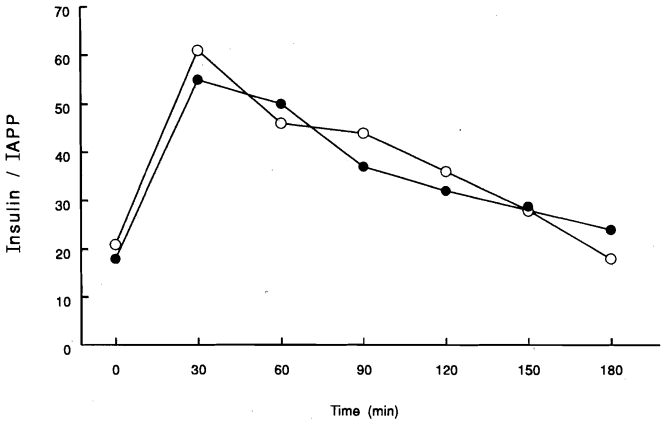

Fig. 2. Mean insulin/islet amyloid polypeptide (IAPP) ratios at each time point during an oral glucose tolerance test before (○) and after (●) treatment with dexamethasone

Following dexamethasone treatment serum glucose levels (Fig. 1 A) were significantly different after 90 min (5.8 ± 0.5 vs 8.3 ± 1.3 mmol/l, $p < 0.05$), 120 min (5.0 ± 0.3 vs 7.3 ± 0.9 mmol/l, $p < 0.05$) and 150 min (4.9 ± 0.3 vs 6.1 ± 0.7 mmol/l, $p < 0.05$). Serum insulin levels (Fig. 1 B) were significantly different after 30 min (408 ± 63 vs 708 ± 98 pmol/l, $p < 0.01$), 60 min (433 ± 99 vs 720 ± 143 pmol/l, $p < 0.05$), 90 min (379 ± 154 vs 549 ± 170 pmol/l, $p < 0.05$) and 120 min (211 ± 50 vs 371 ± 76 pmol/l, $p < 0.01$). Serum IAPP levels (Fig. 1 C) were significantly different at 0 min (3.0 ± 0.5 vs 5.5 ± 0.3 pmol/l, $p < 0.05$), after 30 min (7.5 ± 0.9 vs 15.7 ± 4.0 pmol/l, $p < 0.05$), after 60 min (7.5 ± 0.9 vs 16.8 ± 2.4 pmol/l, $p < 0.05$) and after 90 min (9.3 ± 1.7 vs 18.4 ± 3.5 pmol/l, $p < 0.05$).

The area under the curve (AUC) representing above-basal levels of insulin during OGTT increased significantly following dexamethasone treatment from 48132 ± 9736 pmol · l^{-1} · 3 h^{-1} to 82230 ± 14846 pmol · l^{-1} · 3 h^{-1} ($p < 0.05$), the AUC of IAPP from 1308 ± 183 to 2448 ± 501 pmol · l^{-1} · 3 h^{-1} ($p < 0.05$). The overall insulin/IAPP molar ratios calculated from the AUCs for the entire 3 h of OGTT remained unchanged (42 ± 5 vs 40 ± 4). The insulin/IAPP molar ratios at each time point during OGTT are shown in Figure 2. In short, the insulin/IAPP molar ratios increased significantly from baseline, peaked after 30 min ($p < 0.05$), then declined towards initial values. This kinetic pattern was not changed by dexamethasone treatment.

Following dexamethasone treatment the insulin sensitivity index (SI) calculated using results from FSIGT decreased significantly from 6.5 ± 1.3 to 4.1 ± 1.0 μU · ml^{-1} · min^{-1}, $p < 0.05$.

Discussion

Amyloid deposition in pancreatic islets of patients with Type 2 diabetes and the evidence for co-secretion of insulin and IAPP, the major protein component of these deposits, have led to widespread speculation about the role of IAPP in the pathogenesis of Type 2 diabetes. It remains unclear whether local formation of amyloid is caused by overproduction of IAPP or simply reflects a secondary manifestation of beta-cell dysfunction. Animal experiments revealed a marked increase of beta-cell IAPP immunoreactivity in cats with impaired glucose tolerance, but lack of immunoreactivity of IAPP in beta-cells of overtly diabetic cats [25]. In histopathological studies in Type 2 diabetic patients it has been shown that a decrease of beta-cell IAPP immunoreactivity is accompanied by a marked increase of amyloid deposits [3]. Although these are interesting findings both studies rely on non-quantitative data. An overproduction of IAPP in the OGTT in obese subjects with normal or impaired glucose tolerance was found by several groups [11, 12, 26]. Also in agreement with other groups a relative decrease of IAPP-secretion during the OGTT [11, 12] was found in subjects with Type 2 diabetes, which implies that IAPP-secretion is impaired compared to that of insulin. These findings are in agreement with a study in rats [27], where a reduced IAPP response, without a significant change in insulin response, was seen after treatment with a borderline diabetogenic dose of streptozotocin. A possible change in the insulin/IAPP ratio was therefore thought to be of importance in the pathogenesis of the diabetic state.

Glucocorticoids increase hepatic glucose production and peripheral resistance to the action of insulin – both hallmarks of Type 2 diabetes [28]. Following glucocorticoid treatment, diabetes may develop as a consequence of insufficient compensatory insulin production [28]. Treatment with dexamethasone, therefore, leads to a metabolic condition, which may serve as a model of the development of Type 2 diabetes. Recently two studies have been carried out to investigate the effect of dexamethasone treatment on insulin and IAPP content of pancreatic tissues of rats [21, 22]. Bretherton-Watt et al. [21] demonstrated a 16-fold increase of IAPP mRNA, but only a four-fold increase of insulin mRNA in pancreatic tissues of rats treated with dexamethasone. O'Brien et al. [22] investigated IAPP and insulin secretion from glucose-perfused pancreatic tissues in rats treated with dexamethasone, fed control rats, glucose-treated rats and in fasted rats. In all groups the insulin/IAPP ratio rose sharply and peaked immediately after glucose stimulation. In fasted and fed control rats the insulin/IAPP ratio remained significantly elevated compared with dexamethasone and glucose-treated rats. The authors speculated that increased relative IAPP secretion following dexamethasone treatment may lead to islet amyloid formation and that these findings are consistent with the hypothesis that the production of IAPP and insulin are differently regulated in the beta cell.

In agreement with the results of O'Brien et al. [22] we also observed a change in the insulin/IAPP ratio during the OGTT. The insulin/IAPP ratio rose sharply and peaked 30 min after glucose ingestion and then declined towards initial values. This change in the insulin/IAPP ratio during the OGTT may be caused by a different regulation of these hormones due to different IAPP content within the secretory granules which is released in varying proportions as recently proposed by O'Brien et al. [22]. However, we cannot exclude that the changed insulin/IAPP ratio may simply reflect a different metabolic clearance rate of insulin and IAPP. From studies in patients on haemodialysis and

from observations from Sowa et al. [16], it is assumed that IAPP is cleared predominantly by the kidneys whereas insulin is cleared predominantly by the liver.

In contrast to the studies in rats the kinetics of insulin and IAPP secretion were not changed by dexamethasone treatment in the study. Although the doses used in the studies in rats were higher than those used in this study, we still observed a significant effect on glucose metabolism. We found a significant decrease in insulin sensitivity, a significant increase in insulin secretion and a worsening of glucose tolerance. If a change in the insulin/IAPP ratio would be of importance to induce changes in glucose metabolism, we should have seen it under our experimental conditions.

In conclusion, dexamethasone induced a decrease in insulin sensitivity and an increase in insulin secretion during OGTT. However, in contrast to previous animal experiments, we did not find a change in the insulin/IAPP ratio before and after dexamethasone treatment. At least under our experimental conditions of low-dose corticosteroid treatment, a change in the insulin/IAPP ratio does not seem to be responsible for changes in glucose metabolism as proposed in animal studies [21, 22] and in patients with Type 2 diabetes [11, 12].

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