Insulin is necessary for the hypertrophic effect of cholecystokinin-octapeptide following acute necrotizing experimental pancreatitis

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Abstract

AIM: In previous experiments we have demonstrated that by administering low doses of cholecystokinin-octapeptide (CCK-8), the process of regeneration following L-arginine (Arg)-induced pancreatitis is accelerated. In rats that were also diabetic (induced by streptozotocin, STZ), pancreatic regeneration was not observed. The aim of this study was to deduce whether administration of exogenous insulin could in fact restore the hypertrophic effect of CCK-8 in diabetic-pancreatitic rats.

METHODS: Male Wistar rats were used for the experiments. Diabetes mellitus was induced by administering 60 mg/kg body mass of STZ intraperitoneally (i.p.), then, on d 8, pancreatitis was induced by 200 mg/100 g body mass Arg i.p. twice at an interval of 1 h. The animals were injected subcutaneously twice daily (at 7 a.m. and 7 p.m.) with 1 µg/kg of CCK-8 and/or 2 IU mixed insulin (300 g/L short-action and 700 g/L intermediate-action insulin) for 14 d after pancreatitis induction. Following this the animals were killed and the serum amylose, glucose and insulin levels as well as the plasma glucagon levels, the pancreatic mass/body mass ratio (pm/bm), the pancreatic contents of DNA, protein, amylase, lipase and trypsinogen were measured. Pancreatic tissue samples were examined by light microscopy on paraffin-embedded sections.

RESULTS: In the diabetic-pancreatitic rats treatment with insulin and CCK-8 significantly elevated pm/bm and the pancreatic contents of protein, amylase and lipase vs the rats receiving only CCK-8 treatment. CCK-8 administered in combination with insulin also elevated the number of acinar cells with mitotic activities, whereas CCK-8 alone had no effect on laboratory parameters or the mitotic activities in diabetic-pancreatitic rats.

CONCLUSION: Despite the hypertrophic effect of CCK-8 being absent following acute pancreatitis in diabetic-rats, the simultaneous administration of exogenous insulin restored this effect. Our results clearly demonstrate that insulin is necessary for the hypertrophic effect of low-doses of CCK-8 following acute pancreatitis.

INTRODUCTION

We have previously demonstrated that the administration of low doses of cholecystokinin-octapeptide (CCK-8) accelerated the processes of regeneration following L-arginine (Arg)-induced pancreatitis[1] and this was not observed in rats that were also diabetic[2]. The most significant difference in the regeneration was observed after two wk of CCK-8 treatment[2]. In addition, the histologic examination revealed that the majority of hypertrophized pancreatic acinar cells were found surrounding the enlarged islets of Langerhans following CCK-8 administration. It appears that the close proximity of the islets of Langerhans functions to protect the acinar cells as well as accelerate the regenerative process during Arg-evoked pancreatic tissue damage. A reason for this may be due to the interaction of acinar and islet cells. The exocrine and endocrine pancreas possesses a multitude of complex anatomical and functional interrelations[3]. It is well documented that intact islets of Langerhans are necessary for normal pancreatic exocrine function[4], and so we set out to investigate whether the administration of exogenous insulin could restore the hypertrophic effect of CCK in diabetic-pancreatitic rats.

MATERIALS AND METHODS

Male Wistar rats weighing 250-300 g were divided into five groups. The animals were kept at a constant room temperature of 25 °C with a 12-h light-dark cycle, and were allowed free access to water and standard laboratory chow (Biofarm, Zagyvaszántó, Hungary). Rats in group D (diabetic -control group) were injected with 60 mg/kg body mass of streptozotocin (Zanosar®, The Upjohn Company, Kalamazoo, MI) intraarteritely (i.p.). In group DP (diabetic and pancreatic) the rats received STZ as in group D, and on d 8, pancreatitis was induced by 200 mg/100 g body mass Arg (Sigma, St. Louis, MO) i.p. twice at an interval of 1h. In group DPC, apart from being given Arg and STZ, the rats were also administered 1 µg/kg of CCK-8 (synthesized by Botond Penke, Department of Medical Chemistry University of Szeged) subcutaneously (s.c.) twice daily (at 7 a.m. and 7 p.m.). In group DPI, besides being administered Arg and STZ, the rats received 2 IU mixed insulin (300 g/L short-action and 700 g/L intermediate-action insulin, HUMULIN M3®, Lilly Hungária Kft, Hungary) s.c. twice
daily (at 7 a.m. and 7 p.m.). In group DPCI, diabetes and pancreatitis were induced as in group DP and the rats were administered CCK-8 and mixed insulin as mentioned before. The animals were killed in the morning by exsanguination through the abdominal aorta 14 d after pancreatitis induction. The serum amylase, glucose, insulin and plasma glucagon levels, pancreatic mass/body mass ratio (pm/bm), the pancreatic contents of DNA, protein, amylase, lipase and trypsinogen were measured[2]. Pancreatic tissue samples were examined by light microscopy on paraaffin-embedded sections. Results were expressed as means±SE. Statistical analysis was performed by using ANOVA. P values <0.05 were accepted as significant.

RESULTS

In diabetic groups D, DP, and DPC, the serum glucose levels (22.5±4.1 mmol/L, 29.5±4.0 mmol/L and 29.0±4.7 mmol/L respectively) were significantly elevated and the insulin levels (0.70±0.30 U/L, 0.35±0.09 U/L and 0.20±0.05 U/L respectively) were significantly lower vs those in insulin-treated groups DPI and DPCI (14.5±2.3 mmol/L and 12.9±2.8 mmol/L; 401±38 U/L and 138±35 U/L, respectively). These results clearly indicated the presence of diabetes mellitus in the animals studied and the efficacy of insulin treatment. There were no significant differences in plasma glucagon and serum amylase levels in group D as compared with any of the other groups. In groups DPI and DPCI, pm/bm (2.98±0.21 mg/g, 3.58±0.11 mg/g, respectively) was significantly elevated vs group DP (2.09±0.26 mg/g). However, no significant differences were observed in pm/bm between group DPC and group DP (2.62±0.26 mg/g and 2.09±0.26 mg/g, respectively). In group DPCI (insulin- and CCK-8-treated diabetic-pancreatic rats), the pancreatic protein content (250±35 mg/p) was significantly elevated vs groups DP and DPC (147±10 mg/p and 148±10 mg/p, respectively). In groups DPI and DPCI (insulin-treated rats), the pancreatic amylase content (3.94±0.28 U/p, 10.50±2.93 U/p, respectively) was significantly elevated vs groups DP and DPC (158±4 U/p and 182±25 U/p, respectively). In group DPCI, pancreatic amylase content (10.50±2.93 U/p) was significantly elevated vs group DPI (3.94±0.28 U/p). No significant differences were found in the pancreatic DNA and lipase contents between group D and any other groups. In group DPCI, the pancreatic trypsinogen content was significantly elevated vs group DPI (976±132 IU/p and 592±63 IU/p, respectively). However, no significant differences were observed in the pancreatic trypsinogen content between group DPC (655±36 IU/p) and DP (776±48 IU/p). Histological examination revealed signs of chronic inflammation in diabetic-pancreatic rats, where acute inflammatory cells had been replaced by interstitial tissues, mononuclear cells and fibroblasts. Histological examination did not show any differences between groups DPC and DP. In group DPCI, a more intense mitotic activity was observed vs group DPC due to the effect of insulin (Table 1).

DISCUSSION

The intraperitoneal administration of high doses of Arg induces selective pancreatic acinar cell damage without any morphological change to the islets of Langerhans[5]. STZ has been reported to be specifically toxic to the β-cells of the islets of Langerhans and to induce a dose-dependent and irreversible diabetes in rats without any morphological change to the exocrine pancreas[6]. These models seemed to be suitable for studying the correlation between diabetes mellitus and pancreatitis. The role of insulin in the process of spontaneous and CCK-8-promoted pancreatic regeneration following acute pancreatitis has not yet been characterized in detail. The interesting finding which showed that perinsular acini remained intact during Arg-induced-pancreatitis as well as a lack of the hypertrophic effect of CCK in diabetic rats prompted us to continue studies on the effects of insulin in the process of pancreatic remodelling. Our hypothesis stated that insulin was necessary for the regenerative effect of CCK-8, therefore, we evoked diabetes and pancreatitis in rats as described earlier[7] and then, in the diabetic rats insulin was administered by s.c. injections. The elevation of serum insulin level and the diminution of serum glucose level, clearly showed the efficacy of the insulin treatment. CCK-8 could only elevate pw/bw, the pancreatic contents of protein, amylase and lipase in the presence of insulin. Moreover, CCK-8 could also increase the number of acinar cells with mitotic activity when insulin was administered, but CCK-8 alone had no effect on the laboratory parameters or the mitotic activity in diabetic-pancreatic rats. Lines of evidence demonstrate that both pancreatic secretory and growth processes are (at least partially) under the control of pancreatic islet hormones. Hypoinsulinemia was known to cause pancreatic atrophy and fat infiltration of the exocrine pancreas in guinea pigs[7]. In contrast to this, endogenous and exogenous insulin evoked an increase in pancreatic enzyme synthesis and growth[8]. These direct (via acinar insulin receptors) and indirect (influence on CCK receptors) effects of insulin are well understood. It was also demonstrated that insulin binding to its receptors on the pancreatic acini could be correlated with the subsequent stimulation of protein synthesis[9]. Another indirect observation was that anti-insulin serum completely blocked the CCK-8-stimulated pancreatic secretion in rats[10]. It further suggests that endogenous insulin is necessary for the stimulatory action of CCK-8 on pancreatic exocrine secretion and growth[10]. The present study proves our hypothesis that insulin is indeed necessary for the hypertrophic effect of CCK-8 following acute necrotizing experimental pancreatitis.

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