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Secretory and electrophysiological characteristics of insulin cells from gastrectomized mice: Evidence for the existence of insulinotropic agents in the stomach

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Abstract

Mice were subjected to gastrectomy (GX) or sham operation (controls). Four to six weeks later the pancreatic islets were isolated and analysed for cAMP or alternatively incubated in a Krebs-Ringer based medium in an effort to study insulin secretion and cAMP accumulation in response to glucose or the adenylate cyclase activator forskolin. Freshly isolated islets from GX mice had higher cAMP content than islets from control mice, a difference that persisted after incubation for 1 h at a glucose concentration of 4 mmol/l. Addition of forskolin to this medium induced much greater cAMP and insulin responses in islets from GX mice than in islets from control mice. In contrast, the insulin response to high glucose (16.7 mmol/l) was much weaker in GX islets than in control islets. Glucose-induced insulin release was associated with a 2-fold rise in the cAMP content in control islets. Surprisingly no rise in cAMP was noted in GX islets incubated at high glucose. Capacitance measurements conducted on isolated insulin cells from GX mice revealed a much lower exocytotic response to a single 500 ms depolarisation (from −70 mV to zero) than in control insulin cells. Addition of cAMP to the cytosol enhanced the exocytotic response in insulin cells from control mice but not from GX mice. The depolarisation-triggered inward Ca^{2+} current in insulin cells from GX mice did not differ from that in control mice, and hence the reduced exocytotic response following GX cannot be ascribed to a decreased Ca^{2+} influx. Experiments involving a train of ten 500 ms depolarisations revealed that the exocytotic response was prominent in control insulin cells but modest in GX insulin cells. It seems that cAMP is capable of eliciting insulin release from insulin cells of GX mice only when cAMP is generated in a specific microdomain conceivably through the intervention of membrane-associated adenylate cyclases that can be activated by forskolin. The GX-evoked impairment of depolarisation-induced exocytosis and glucose-stimulated insulin release may reflect the lack of a gastric agent that serves to maintain an appropriate insulin response to glucose and an appropriate exocytotic response to depolarisation by raising cAMP in a special glucose-sensitive compartment possibly regulated by a soluble adenylate cyclase.

Keywords: Insulin release; Gastrectomy; Exocytosis; cAMP

1. Introduction

Insulin cells release insulin in response to a rise in blood glucose. The current view is that the uptake of glucose into the insulin cell results in an increased intracellular ATP concentration. The increase in ATP (at the cost of ADP) leads to closure of ATP-dependent K^+-channels, depolarisation of the cell membrane and influx of Ca^{2+} through voltage-dependent Ca^{2+}-channels. The consequent increase in intracellular Ca^{2+} in the vicinity of the Ca^{2+}-channels initiates granule exocytosis and insulin release [1]. Several events in the process of glucose-induced stimulus–secretion coupling are influenced by cAMP. Hence, formation of cAMP from ATP leads to activation of protein kinase A (PKA) [2–4], generating an increase in intracellular Ca^{2+} through a PKA-dependent mechanism [5,6].

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Exocytosis is preceded by PKA-dependent phosphorylation of proteins involved in the transport of insulin granules from the so-called reserve pool to the readily releasable pool [1]. Moreover, cAMP acts synergistically with glucose to reduce the activity of ATP-dependent K^+-channels [4], activate L-type Ca^{2+} channels [3], and accelerate several steps in the exocytotic process [2,7]. Forskolin and the incretins, gastric inhibitory peptide (GIP) and glucagon-like peptide 1 (GLP-1), are known to amplify glucose-stimulated insulin release by activating adenylate cyclase in the insulin cells [3]. Although many of the cAMP-evoked events listed above are thought to be mediated by cAMP-activated PKA, recent data have suggested that cAMP enhances insulin secretion also through PKA-independent pathways, involving cAMP-regulated guanine nucleotide exchange factor II (cAMP-GEFII or Epac) [8]. Stimulation of cAMP-GEFII affects Ca^{2+}-induced mobilization of intracellular Ca^{2+} [6] and this is thought to help make secretory granules release-competent [2]. Hence, the role of cAMP in the regulation of glucose-stimulated insulin secretion is complex and not fully understood and if the experimental results seem contradictory at times, it is probably because cAMP acts *via* several different pathways. Indeed, there is evidence for a strict subcellular compartmentalization of cAMP and cAMP-induced actions in other cell types [9–11].

Gastrectomy (GX), in patients as well as in animals, impairs the ability of insulin cells to respond to a glucose challenge with the appropriate release of hormone [12–16]. This has been studied previously in GX mice *in vivo* and in isolated pancreatic islets from GX mice [14]. From these data, we have suggested that the impairment might reflect the loss of a gastric regulatory peptide that serves to enhance the insulin response to oral as well as circulating glucose [14]. Indeed we could show that the impaired insulin response to glucose in GX mice was paradoxically associated with an enhanced responsiveness to cholinergic agents and especially to agents that stimulate cAMP formation [14].

In the present study we have expanded our previous investigation [14] in order to explore further the nature of the GX-induced defects in the insulin secretory machinery, especially with regard to cAMP. To this end, we determined cAMP in freshly isolated islets and studied the insulin response to glucose and to the adenylate cyclase activator forskolin as well as the ability of the islets to accumulate cAMP upon incubation for 1 h in the presence of different concentrations of glucose. In addition, we compared isolated insulin cells from GX mice and control mice with respect to the impact of cAMP on their electrophysiological properties.

2. Materials and methods

2.1. Animals

Twenty-eight female mice of the NMRI strain (B&K Universal, Sollentuna, Sweden) were used in the experiments. They weighed 26.7 g±0.7 before the operation. They were kept on a standard pellet diet (Lactamin, Stockholm, Sweden) and tap water *ad libitum*. Before surgery, they were anaesthetized with mebumal (25 mg per mouse, i.p.). Surgery was performed through a midline abdominal incision with clean but not sterile instruments. No antibiotics were used. Gastrectomy (GX) was performed by resecting the stomach and anastomosing the oesophagus and the duodenum end-to-end. Sham operation consisted of an abdominal midline incision and manipulation of the viscera (laparatomy). The animals were allowed to recuperate for 4–6 weeks before they were subjected to experiments. During this time period suitable after-care was given to the animals to make sure that they suffered no pain or distress. One of the 15 GX mice died (during the first few days after surgery). At the time of removal of the pancreas the GX mice weighed 30.9 g±1.2 (n=14) and the sham-operated mice 32.3 g±0.9 (n=13). The study was approved by the local animal welfare committee (Lund, Sweden).

2.2. Chemicals

Collagenase (fraction CLS 4) was obtained from Worthington Biochemicals, Freehold, NJ, USA. Bovine serum albumin was from ICN Biochemicals, High Wycombe, UK. Isobutylmethylxanthine (IBMX), an inhibitor of phosphodiesterase, and all other drugs and chemicals were from Sigma Chemicals, St Louis, MO, USA or Merck AG, Darmstadt, Germany. Radioimmunoassay kits for insulin determination were obtained from Diagnostika (Falkenberg, Sweden).

2.3. Experimental protocol

2.3.1. Pancreatic islets

Pancreatic islets were isolated by retrograde injection of a collagenase solution via the bile–pancreatic duct [17]. Islets (of similar size) were then collected under a stereomicroscope at room temperature (usually 250 islets per mouse pancreas). In one experiment (shown in Fig. 1) 0.2 mmol/l IBMX was present

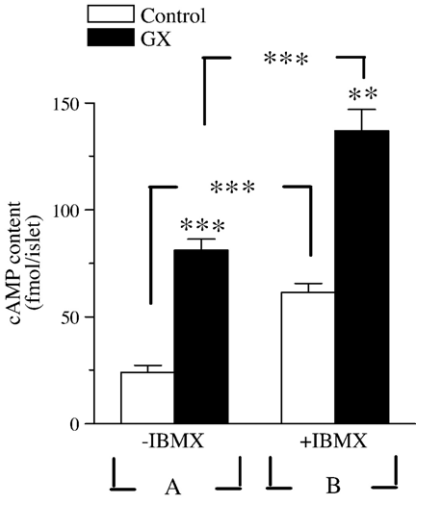

Fig. 1. cAMP content in islets from sham-operated (control) and gastrectomized (GX) mice isolated "ex vivo" in the absence (A) and presence (B) of 0.2 mmol/l IBMX. Means±SEM for four batches of islets; each batch isolated from one mouse. Significant difference between GX and controls **p<0.01; ***p<0.001.