Glucose dependence of insulinotropic actions of pituitary adenylate cyclase-activating polypeptide in insulin-secreting INS-1 cells

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Abstract The cAMP-elevating pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates insulin release in pancreatic B-cells. Here, we have investigated its potentiating action in rat insulinoma INS-1 cells. In intact cells, PACAP-27 (100 nM) stimulated glucose-induced insulin secretion by >60%. Using the patch-clamp technique with single-cell exocytosis monitored as increases in cell capacitance, we observed that at 10 mM and 20 mM extracellular glucose, PACAP-27 acted mainly by a >50% enhancement of depolarization-elicited Ca2+ entry, whereas at low (3 mM) glucose, the predominant effect of the peptide was a twofold increase in Ca2+ sensitivity of insulin exocytosis. The latter effect was mimicked by glucose itself in a dose-dependent fashion. PACAP-27 exerts a prolonged effect on insulin secretion that is dissociated from changes of cytoplasmic cAMP. Whereas an elevation of cellular cAMP content (135%) could be observed 2 min after addition of PACAP-27, after 30 min preincubation with the peptide, cAMP concentrations were not different from basal. Yet, such pretreatment with PACAP-27 stimulated subsequent insulin release by ≈60%. This sustained action is likely to reflect an increased degree of protein-kinase-A-dependent phosphorylation, and inhibitors of the kinase largely prevented the PACAP-mediated effects.

Keywords Cyclic AMP · Exocytosis · GLP-1 · INS-1 cells · Insulin · PACAP

Introduction

The insulinotropic pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide originally isolated in the ovine hypothalamus [31]. The peptide is expressed in several tissues throughout the body, including the CNS, the adrenal medulla and the gastrointestinal tract [6, 39]. In the pancreas, it localizes to intrapancreatic nerve ganglia and single nerves in the exocrine parenchyma, as well as around blood vessels and to endocrine islets [16, 40]. PACAP is structurally related to vasoactive intestinal polypeptide (VIP) and glucagon-like peptide 1(7–36)amide (GLP-1), particularly at the N-terminal [26, 27]. Like these peptide hormones, PACAP has been demonstrated to potentiate glucose-stimulated insulin release in vitro [27, 40] and in vivo in mice [16] and in humans [13]. Two forms of the peptide have been identified, PACAP-38 and PACAP-27; the latter representing the amidated N-terminal 27 residues of the full-length hormone [32]. The two variants stimulate insulin secretion equipotently in both isolated islets and perfused pancreases [14, 40, 41].

The effects of PACAP are mediated via three different receptors: the PACAP type 1, the VIP1/PACAP and the VIP2/PACAP receptors [7]. In mouse and rat pancreas, the presence of type 1 and VIP2/PACAP receptors has been demonstrated [14]. The insulinotropic effect of PACAP is believed to occur mainly by generation of cAMP in the B-cells [27]. cAMP potentiates insulin exocytosis by both protein-kinase-A- (PKA-) dependent and PKA-independent pathways [34, 36]. Stimulation of the cAMP/PKA system has been shown to improve the defect insulin secretion in GK-rats, an established animal model for type-2 diabetes [1]. In addition, recent PAC1-receptor knock-out studies indicate a role for PACAP in glucose-induced insulin secretion [25]. Elucidating the mechanisms whereby PACAP stimulates insulin secretion is interesting not only for obtaining a better understanding of type-2 diabetes development, but also for potentially improving the treatment of the disease. For example, glucagon-like peptide 1(7–36)amide (GLP-1), which is
believed to stimulate insulin secretion by similar actions [12, 18], has attracted much attention as a possible therapy in non-insulin-dependent diabetes mellitus (NIDDM) [23].

Here we have used the patch-clamp technique to explore the effects of PACAP-27 on electrical activity and Ca2+-induced exocytosis in insulin-releasing clonal INS-1 cells, a well-established model of insulin secretion from B-cells. In combination with insulin and cAMP measurements by RIA we have aimed to clarify the cellular and molecular mechanisms whereby PACAP-27 augments insulin release at different extracellular glucose concentrations and how these mechanisms compare with the actions of GLP-1.

Materials and methods

Cells

INS-1 cells (kindly donated by C.B. Wollheim, Geneva, Switzerland; 2.5 × 106 cells/well) were cultured in plastic flasks at 37°C in 5% CO2-95% air in RPMI medium supplemented with 50 μM mercaptoethanol (Gibco BRL, Paisley, UK), 2.06 mM l-glutamine (Life Technologies, Täby, Sweden), 10% fetal bovine serum, 100 units ml−1 penicillin and 0.5 mg ml−1 streptomycin (all from Kebo Laboratory, Spånga, Sweden). The cells were divided after 7 days (about 75% confluence) and for electrophysiology the cells were put on plastic Petri dishes and maintained in tissue culture for 1–2 days prior to the experiments. The cells used were from passages 82–90.

Electrophysiology

The plastic Petri dishes were used as the experimental chamber with a plastic insert to reduce the volume to approximately 0.5 ml, and were continuously perfused at a rate of 2 ml min−1. All experiments were conducted at 31–33°C. Patch electrodes were made from borosilicate glass capillaries, coated with Sylgard to their tips and fire-polished. The pipette resistance was 3–6 MΩ when the pipettes were filled with the intracellular solutions specified below. The zero-current potential of the pipette was adjusted with the pipette in the bath. The experiments were performed using EPC7 or EPC9 patch-clamp amplifiers and the software Pulse (version 8.3 or later; HEKA Elektronik, Lambrecht/Merseyside, UK) as indicated in text or legends. In the voltage-clamp experiments 20 mM NaCl was replaced (equimolar) with TEACl, to block outwardly rectifying K+ currents [37] that otherwise obscure the depolarization-evoked Ca2+ currents. The pipette solution contained (in mM): 76 K2SO4, 10 KCl, 10 NaCl, 1 MgCl2, 5 HEPEs (pH 7.35 with KOH). To enhance resolution of the depolarization-evoked Ca2+ currents in the voltage-clamp experiments, K2SO4 and KCl were replaced with equimolar amounts of Cs2SO4 and CsCl, and CsOH was used to set pH at 7.35. Electrical contact with the cell interior was established by using the pore-forming antibiotic amphotericin B (Sigma), with a final concentration of 0.24 mg/ml. Perforation required a few minutes and was considered satisfactory when the series conductance (Gseries) was stable and above 40 nS (25 MΩ). The cells were preincubated with PACAP-27 (100 nM), GLP–1 (100 nM) or the PKA inhibitor Rp-cAMPS, at 37°C for 30 min as indicated, and the recordings were then made within 60 min.

Solutions

The standard extracellular medium was composed of (in mM): 138 NaCl, 5.6 KCl, 2.6 CaCl2, 1.2 MgCl2, 5 HEPEs (pH 7.4 with NaOH) and was supplemented with d-glucose and PACAP-27 (100 nM) or GLP-1 (100 nM; both from Peninsula Europe, Merseyside, UK) as indicated in text or legends. In the voltage-clamp experiments 20 mM NaCl was replaced (equimolar) with TEACl, to block outwardly rectifying K+ currents [37] that otherwise obscure the depolarization-evoked Ca2+ currents. The pipette solution contained (in mM): 76 K2SO4, 10 KCl, 10 NaCl, 1 MgCl2, 5 HEPEs (pH 7.35 with KOH). To enhance resolution of the depolarization-evoked Ca2+ currents in the voltage-clamp experiments, K2SO4 and KCl were replaced with equimolar amounts of Cs2SO4 and CsCl, and CsOH was used to set pH at 7.35. Electrical contact with the cell interior was established by using the pore-forming antibiotic amphotericin B (Sigma), with a final concentration of 0.24 mg/ml. Perforation required a few minutes and was considered satisfactory when the series conductance (Gseries) was stable and above 40 nS (25 MΩ). The cells were preincubated with PACAP-27 (100 nM), GLP–1 (100 nM) or the PKA inhibitor Rp-cAMPS, at 37°C for 30 min as indicated, and the recordings were then made within 60 min.

Insulin measurements

The cells were washed twice in a HEPEs buffer containing (in mM): 125 NaCl, 5.9 KCl, 1.28 CaCl2, 1.2 MgCl2, 5 HEPEs, 3 glucose and 0.1% human serum albumin (pH 7.36), and preincubated for 30 min at 37°C in 200 μl of the incubation medium with or without PACAP-27 (100 nM) or GLP-1 (100 nM). After preincubation, the cells were washed and then incubated for 60 min at 37°C in 200 μl of medium, with or without PACAP-27 or GLP-1. Following this, 150 μl of the medium was centrifuged at 350 g for 5 min, aliquots of 50 μl were frozen at −20°C and saved for analysis of insulin content by double antibody RIA (Linco Research, St Charles, Mo., USA), using guinea pig anti-porcine insulin, mono-125I-insulin, and, as standard, rat insulin. The antigen-antibody complexes were dissociated by the double-antibody technique.

Cellular cAMP content

INS-1 cells were washed twice in a HEPEs buffer and preincubations (30 min) were carried out as described above. They were then incubated for 2 min under the indicated experimental conditions, before reactions were stopped by the addition of ice-cold ethanol reaching a final concentration of 65%, and the cells were scraped off with a rubber policeman. After being washed twice in 65% ice-cold ethanol, the extracts were centrifuged at 2000 g at +4°C for 15 min, transferred to fresh test-tubes, evaporated at +60°C under a stream of nitrogen and then stored at −20°C until analysis for protein content by the Lowry method [29] and for cAMP by RIA (Amersham, UK), using a rabbit antisuccinyl AMP serum, cyclic 2-succinyl-3-[125I]methyl ester as tracer, and cAMP as standard. The antigen-antibody complexes were dissociated by the double-antibody technique.

Data analysis

Data are presented as mean values ±SEM of n cells or experiments as appropriate. In electrophysiology experiments, for any change in the composition of the extracellular medium, e.g. increase in glucose concentration, addition of PACAP-27 or GLP-1, the effects were measured in the steady-state, which occurred within 4–6 min. Statistical significances were evaluated using experimental values. Paired Student’s t-test was used when comparing results obtained in the same cell, and ANOVA with subsequent Dunnet’s test when comparing independent groups. Percentages were determined by taking the control value as 100%. Thus, an increase in capacitance...