EVIDENCE FOR A LIMITED ROLE OF NAD(P)H IN THE NUTRITIONAL REGULATION OF GLUCAGON RELEASE: STUDIES WITH MENADIONE AND NH₄Cl

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Several observations suggest that the metabolism of glucose in islet cells may play an essential role in the regulation of glucagon release. A₂-cells possess a high enzymatic capacity for degradation of triose phosphates and, at high glucose concentration, the consumption of oxygen, the utilization and oxidation of glucose and ATP concentration are all increased in A₂-cell rich islets removed from guinea-pigs treated with streptozotocin. In addition, α-ketoisocaproate (KIC), which is a potent insulin secretagogue and is actively metabolized in islet cells inhibits glucagon release in the same manner as an increase in the concentration of glucose.

The substrate-site hypothesis for insulin release is gaining increasing weight and it was proposed that the availability of reduced pyridine nucleotides (NADPH +NADH) may represent one of the key coupling factors in the process of nutrient-induced insulin release by the B-cell. It was recently reported that A₂-cells contained a NADPH-dependent glutathione reductase. The present work was undertaken to evaluate the possible role of reduced pyridine nucleotides in glucagon release from the perfused rat pancreas. The studies were performed using menadione and NH₄⁺, which are potent inhibitors of insulin release. Both agents are thought to act primarily through their ability to lower the concentration of reduced pyridine nucleotides in isolated pancreatic islets. Part of the present work has been reported in abstract form.

Key-words: Arginine; Glucagon; Glucose; Insulin; Menadione; NH₄Cl; Pancreas; Perfusion; Rat.

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MATERIALS AND METHODS

Fed female albino rats were used (mean body weight ± SEM: 2.59 ± 0.7 g; n = 24). The perfusion technique has been detailed previously. The whole pancreas, all adjacent organs being excluded, is perfused through the celiac and mesenteric arteries. The perfusate was composed as follows, in mmol/l: NaCl, 118.5; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; CaCl₂, 2; glucose, 3.3. It was supplemented with dextran (40 g/l, T 70, Pharmacia, Uppsala, Sweden) and bovine albumin (5 g/l, fraction V, Sigma, USA). The medium was constantly equilibrated against a mixture of oxygen and carbon dioxide (95:5, v/v) and entered the pancreas with a pH of 7.4 and a temperature of 37.5°C. The flow rate and perfusion pressure (mean ± SEM, n = 24) were 1.98 ± 0.01 ml/min and 31.5 ± 1.0 mm Hg (4.2 ± 0.13 kPa), respectively. L(+)-arginine, glucose and NH₄Cl (Merck, Darmstadt, FRG) were dissolved in twice distilled water and infused through side-arm syringes at a flow rate of 0.075 ml/min. The increase in osmolality induced by glucose and NH₄Cl were corrected by equivalent decreases in the amount of NaCl in the perfusate (5 to 15 mmol/l). Menadione was first dissolved in dimethylsulfoxide (Me₂SO₄). The same amount of Me₂SO₄ (final concentration in the perfusate: 0.6 g/l/ml) was administered at the same flow rate during the pre-menadione infusion periods. Menadione was always infused 15 min before the glucose stimulus, since it was previously shown that the inhibitory action of the drug on insulin release was more efficient in that condition than in B-cells already exposed to glucose.

Samples of the effluent were collected at 1-min intervals in chilled tubes containing 2,000 KIU aprotinin (Trasylol) and frozen at −25°C until time of assay. Glucagon (0.2 ml aliquots) and insulin (0.01 to 0.2 ml aliquots) were measured at the times shown on the figures with individual radioimmunoassays for these hormones. The tracers were ¹²⁵I-glucagon (375 to 490 μCi/μg, CNTS, Paris, France; NEN, Boston, USA) and ¹²⁵I-insulin (124 to 132 μCi/μg, Sorin, Italy). The standards were beef-pork glucagon (Lot 234-13-161-1, Lilly, Indianapolis, USA) and rat insulin (Lot R 170, Novo, Bagsvaerd, Denmark). Glucose was assayed in the pancreatic effluent with a glucose-oxidase method (GOD-PAP, Boehringer, Mannheim, FRG) using an AA-I Technicon analyser.

Rates of glucagon or insulin release were calculated from the areas under the curves. Statistical analyses were conducted using two-tailed paired or non-paired t-tests.

RESULTS

Effects of menadione on glucagon and insulin release in the absence of arginine - In the absence of menadione (control experiments), a rise in glucose concentration (from 3.3 to 11.1 mmol/l) provoked a rapid inhibition of glucagon release (fig. 1, left panel). A significant reascension in glucagon output was observed upon restoration of the low glucose concentration. In the experimental group (fig. 1, right panel), the initial output of glucagon was slightly but not significantly lower than in the control group. The infusion of menadione had little effect upon the rate of glucagon release seen in the presence of glucose 3.3 mmol/l. The tendency towards an initial reduction in glucagon output (tab. 1, line 2 vs 3) could not be dissociated from the spontaneous decline in glucagon release often seen at the