Research Article

Dynamic insulin secretion from perifused rat pancreatic islets

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Abstract. Insulin secretion from isolated pancreatic islets of 8- to 12-day-old rats was investigated in a dynamic in vitro (perifusion) system. The aims of the study were (i) to describe a carefully controlled in vitro method to study the mechanism of insulin secretion and to analyse the effects and dynamic interactions of bioactive compounds on isolated rat pancreatic islets, (ii) to validate the method by comparing fundamental data on the functions of the islets obtained with this method to those collected with other techniques; and (iii) to find novel features of the control of insulin secretion. The method was carefully designed to maintain the functional capacity of the explanted cells. A functional standardization system was elaborated consisting of (i) analysis of the changes in the basal hormone secretion of the cells; (ii) evaluating responses to a standard, specific stimuli (50 mM glucose for 3 min); (iii) determining the alteration of the momentary size of the hormone pool with responses to KCl; and (iv) direct determination of the total intracellular hormone content from the extract of the column. The technique provides accurate quantitative data on the dynamic responses to biologically active compounds that act directly on the pancreatic islets. The islets maintained their full responsiveness for up to 7 days, and responses as close as in 1-min intervals could be distinguished. A linear dose-response relationship was found on the glucose-induced insulin release in case of 3-min stimulation with 4 and 500 mM of glucose (lin-log graph). Utilizing this method, we showed that no desensitization to glucose-induced insulin release can be observed if the responsiveness of the cells is properly maintained and the parameters of the stimulation are carefully designed. Exposure of the explanted islets to 10 μM acetylcholine or 30 mM arginine (Arg) induced a transitory elevation of insulin release similar in shape to that experienced after glucose stimulation. Nor- epinephrine (NE), dopamine (DA) and somatostatin (SS) did not induce any detectable alteration on the basal insulin secretion of the islets. However, 100 nM SS given together with 50 mM glucose, 30 mM Arg or 10 μM acetylcholine significantly reduced the insulin-releasing effect of these substances (by 75.5, 71.5 and 72.5%, respectively). At the same time, SS did not alter the insulin response of the islets to 100 mM elevation of K+ concentration. SS also inhibited glucose-induced insulin release in a dose-dependent way (ED50 = 22 nM). A similar dose-dependent inhibitory effect on glucose-induced insulin release was found with NE (ED50 = 89 nM) and DA (ED50 = 2.2 μM). γ-Aminobutyric acid (GABA) did not influence insulin release under similar circumstances.

Key words. Pancreatic islets; insulin; perifusion; secretion kinetics.

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Understanding the control and mechanism of insulin secretion from the pancreatic islets is of increasing importance both theoretically and in clinical practice. The regulation of pancreatic islets is based on a coordinated interplay of several factors, including various nutrients, gastrointestinal and pancreatic hormones as well as the autonomic nervous system and its transmitters.

The potential therapeutic utilization of isolated pancreatic islet transplant also calls for a better understanding of the islet physiology. In vivo observations and experiments provided invaluable contribution to our knowledge of the physiology of the pancreatic islets. However, metabolic feedback, unknown mechanisms of metabolism and absorption of test compounds prevent studying the direct effects and interactions of various bioactive compounds on the cells of the pancreatic islets. For instance, glucose has a stronger effect on the insulin release if it is taken per os in comparison to its parenteral application [1, 2]. The physiological, pulsatile stimulation and delicate timing of the dynamic responses are also difficult to follow under in vivo circumstances. Although, based on observation of pituitary cells, the response time of the release of hormones from peptide-secreting endocrine cells is expected to be well within 1 min [3], most of the experiments targeting the functions of pancreatic islets are based on much longer expected response times.

Dynamic in vitro bioassays meet these requirements. One form of this method is the perfusion technique, which utilizes isolated tissues perfused with a continuous stream of fresh tissue culture media. This system is especially suitable for the study of the direct effect of the various substances and their combinations on hormone release. A carefully controlled, well-designed system, described earlier by Csernus and Schally [3] in detail to study anterior pituitary cells, had to be adjusted to the conditions of pancreatic islets. To check the validity of the perfusion system in studying isolated rat pancreatic islets, our results were compared with results collected from in vivo or static in vitro investigations (batch technique).

The islets of Langerhans are integrated into the neuroendocrine system. Several components of this system affect insulin release by either enhancing or inhibiting it. Controversial data were found in the literature on the specific role of several bioactive compounds. These data were based on in vivo observations or experiments with perfused pancreatic slices [4], islets [5–7] or isolated B cells [8–10]. In particular, the dose dependency of glucose [11] or arginine [6, 8, 12]-stimulated insulin release, biphasic insulin release [11, 13], somatostatin inhibition of glucose-induced insulin release [14, 15] and insulin desensitization [16, 17] were investigated. Several inconsistent data were published as well on the cysteamine depletion of somatostatin from the D cells of the pancreatic islets [18], the pulsatile nature of the insulin secretion [19, 20] as well as on the effects of acetylcholine [15, 21–25], dopamine [26], norepinephrine [21, 27–34] and GABA [35] on the insulin secretion. Some of these discrepancies stem from neglected differences in the experimental setup. It has been shown that, depending on the circumstances in culture, profound alteration of the inositol phosphate-related intracellular signalling system can occur in the islet cells [15, 36]. Our goals were (i) to describe a carefully controlled in vitro bioassay with proper, functional standardization that provides accurate, quantitative data on the dynamic responses of explanted pancreatic islets, (ii) to investigate the possibilities and limitations of this method, (iii) to check the validity of the results obtained from this system by comparing them with data of other in vivo or static in vitro investigations and (iv) to study the direct effects of biologically active compounds on isolated pancreatic islets investigated in a well-defined environment.

Materials and methods

Chemicals. Medium-199 (M-5017), BSA (A-7906), Sephadex G-10 (G-10-120) and Gentamycin (G-3632) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Collagenase was a product from Serva (Heidelberg, Germany, no. 17449). Hanks’ bovine serum albumin (BSA) solution contained 1 g/l BSA (Sigma A-7906) in Hanks’ solution. Trasylol was produced by Bayer (Leverkusen, Germany). Other organic and inorganic chemicals were obtained from Merck (Darmstadt, Germany; purity p.a.). All solutions were made with pyrogen-free water prepared in a Barnstead EASYpure RF system.

Islet preparation. Eight- to twelve-day-old Wistar rats of both sexes were used. The animals were born in the animal facility of our laboratory and kept in a standard environment (L:D = 12:12, lights on 7 a.m.; temperature 21 ± 1 °C; food: Altromin 1316 and water were available ad libitum). Animals were sacrificed by decapitation, and pancreata of four animals were removed, cut into approximately 1-mm pieces and transferred to 5-ml glass tube containing 1 mg/ml collagenase in 2 ml of ice-cold Hanks/BSA. The tubes were hand-shaken vigorously for 5 min. The suspension was transferred to 30 ml of ice-cold Hanks/BSA. The islets were sedimented for 5 min and resuspended in 30 ml of Hanks/BSA. The sedimentation was repeated twice. The islets, free from exocrine tissue pieces, were collected with a glass capillary pipette under a preparation microscope.

Perfusion. Perfusion analysis of isolated rat pancreatic islets was performed in a system described earlier in detail [3]. Briefly, about 300 isolated pancreatic islets