THE EFFECT OF CYCLOHEXIMIDE AND 2-DEOXYGLUCOSE ON THE DIPHASIC PATTERN OF INSULIN SECRETION

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The fact has been well documented that the isolated perfused rat pancreas will secrete insulin in response to an adequate glucose stimulus. In previously reported experiments from this laboratory, a diphasic pattern of insulin secretion, in response to a constant glucose stimulus, has been described. It was suggested that the early phase of release represented only the release of prestored hormone, whereas the second release phase constituted the secretion of both prestored and newly synthesized hormone, and that the contribution of this newly synthesized insulin became more pronounced as a function of time (up to the 60-min time period studied). The purpose of this paper is to further investigate factors controlling both phases of secretion. In particular, relative sensitivities of each phase to glucose will be reported, as well as the effect of cycloheximide on both phases.

The inhibitory action of 2-deoxyglucose on both phases of insulin secretion

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will also be described, such that one may gain insight as to the effect of the glycolytic pathway on secretion and synthesis of insulin.

METHODS

In the experiments presented in this paper, as in those previously reported from this laboratory, male Long-Evans rats which weighed approximately 250 g were anesthetized with sodium pentobarbital and the portion of the gastrointestinal tract which receives its blood supply from the celiac axis was removed. This includes the pancreas with the adjacent proximal portion of the duodenum, the spleen, and the stomach. The portal vein and the celiac axis were then connected to the respective venous and arterial cannulae of the perfusion apparatus.

The perfusion medium consisted of the ionic solution described by Umbreit et al. To this was added an appropriate amount of 25% human albumin from Cutter Laboratories in order to produce a final albumin concentration of 4%, which served as the colloid source. Each pancreas was then perfused by recycling the medium for a period of 10 min, during which time the temperature was adjusted to 37-38 °C and the rate of perfusate flow was adjusted to 10 ml/min.

At the end of the stabilization period, recycling was stopped and the entire venous outflow was collected as a function of time. This was done by continually collecting the portal vein outflow in a series of graduated test tubes for periods ranging from 30 sec to 3 min. The first two samples, 0-1 and 1-2 min, served as baseline controls. At the end of the 2nd min, a continuous glucose infusion (either 150, 300, or 600 mg %) was begun which lasted until 90 min, when it was discontinued. Then, beginning at 96 min, another glucose infusion of the same concentration was begun and continued until 98 ½ min. In some instances, 2-deoxyglucose (300 or 600 mg %) was added to the perfusate 20 min prior to time zero, and in other cases cycloheximide (1 mg/ml) was added to the perfusate 30 min prior to zero time.

Insulin concentration of the venous effluent from each of the above-described tubes was determined by the radioimmunochemical method of Grodsky and Forsham, using pure rat insulin from Novo Laboratories as the reference. Total insulin secretion was calculated from the measured rates of flow and the insulin concentration of the venous effluent, then reported as nanograms of insulin released per 30 sec. Glucose levels were attained by infusing a known amount at a rate calculated to produce the desired perfusate concentration.

METODOLOGIA

Negli esperimenti descritti nel presente lavoro, come pure in quelli compiuti nel nostro laboratorio e precedentemente riferiti, a ratti maschi Long-Evans del peso di circa 250 g è stata anestetizzata, previa anestesia con pentobarbital sodico, quella parte del tratto gastrointestinal che riceve il suo apporto ematico dal tronco celiaco. Questa comprende il pancreas con l'adiacente porzione prossimale del duodeno, la milza e lo stomaco. La vena porta e il tronco celiaco sono stati poi collegati rispettivamente alle cannule venosa ed arteriosa dell'apparecchio di perfusione.

Il mezzo di perfusione era costituito dalla soluzione ioniaca descritta da Umbreit e Coll., per cui veniva aggiunta un'appropriata quantità di albumina umana al 25% (Cutter Laboratories), onde ottenere una concentrazione finale di albumina del 4%, che fungeva da supporto colloidale. Ogni pancreas veniva poi perfuso facendo ricircolare il mezzo per un periodo di 10 min, durante il quale la temperatura veniva regolata a 37-38 °C e la velocità di flusso del perfusato a 10 ml/min.

Al termine del periodo di stabilizzazione, la ricircolazione veniva arrestata e l'intero efflusso venoso raccolto in funzione del tempo. A ciò si provvedeva raccogliendo di continuo in una serie di provette graduate, per periodi variabili da 30 sec a 3 min, il liquido effluente dalla vena porta. I primi due campioni (0-1 e 1-2 min) servivano quali controlli di base. Alla fine del 2° min si iniziava un'infusione continua di glucosio (150, 300 o 600 mg%), che era protratta fino al 90° min, allorché essa veniva sospesa. Poi, a partire dal 96° min, si iniziava un'altra infusione di glucosio alla stessa concentrazione, continuata fino al 98° min e mezzo. In alcuni casi, al perfusato veniva aggiunto, 20 min prima del tempo 0, 2-desossiglucosio (300-600 mg %) ed in altri, 30 min prima del tempo 0, cicloeximide (1 mg/ml).

In ciascuna di tali provette, la concentrazione di insulina nel liquido venoso effluente raccolto veniva determinata mediante il metodo radioimmunochimico di Grodsky e Forsham, utilizzando come riferimento insulina pura di ratto (Novo Laboratories). La secrezione totale di insulina veniva calcolata in base alle velocità di flusso misurate e alla concentrazione di insulina nel liquido venoso effluente, ed espressa in nanogrammi di ormone liberati in 30 sec. I livelli di glucosio venivano ottenuti infondendo una quantità nota della sostanza ad una velocità calcolata per produrre nel perfusato la concentrazione desiderata.