THE IMMEDIATE INSULIN SECRETORY RESPONSE OF THE ISOLATED PERFUSED RAT PANCREAS TO TOLBUTAMIDE AND GLUCOSE

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1. Introduction

It is well recognized that glucose and tolbutamide induce different immediate insulin secretory responses of pancreatic beta cells [1–4]. The first peak of insulin release appears one to two minutes earlier in response to tolbutamide than in response to glucose [1,2,4]. It may be anticipated, therefore, that islet cells exposed to both glucose and tolbutamide exhibit an immediate insulin secretory response consisting of two separate peaks. Any kind of synergistic action, however, would result in a single possibly exaggerated peak. It has been reported previously that tolbutamide and glucose when administered simultaneously to the pancreas induce an immediate secretory response consisting of one peak only [2,3]. In the present investigation sampling intervals were reduced to 10 sec as compared to 30 sec chosen by other investigators [2] in order to detect rapid changes in insulin release from the isolated perfused pancreas.

2. Experimental

As gifts were supplied: pure rat insulin (Novo GmbH, Mainz), pentobarbital (Knoll AG, Ludwigshafen) and tolbutamide (Farbwerke Hoechst AG, Frankfurt). ^{125}I-labelled insulin was obtained from Farbwerke Hoechst AG, Frankfurt, bovine albumin (Fraction V) from Serva, Heidelberg. All other chemicals of analytical grade were from Merck AG, Darmstadt.

Male albino rats (Winkelmann, Kirchborchen) (approx. 220 g b. wt) were anaesthetized by an intraperitoneal injection of pentobarbital (45 mg/kg b. wt). The pancreas and the adjacent part of the duodenum, the spleen and the stomach were removed according to the method of Grodsky et al. [5]. The abdominal aorta and the portal vein were connected to the arterial and venous cannulae, respectively. The perfusion medium consisted of Krebs–Ringer bicarbonate solution containing 0.2% bovine albumin. It was gassed with 95% oxygen and 5% carbon dioxide which resulted in a pH of 7.4. The temperature was maintained at 37°C. The flow rate was adjusted to 4 ml/min which resulted in a pressure between 45 and 55 mm Hg. The dead space of the perfusion system between the valve and the venous effluent was about 2 ml. The arterial oxygen tension was 450–550 mm Hg, the venous oxygen tension 250–350 mm Hg. After an initial equilibration period of 10 min, designed zero time, the perfusion was switched to a medium containing the respective stimulating agents. The venous effluent was collected at various timed intervals (either 10 sec or 1 min) as described in fig.1.

The immunoreactive insulin was determined by the method of Zaharko and Beck [6].

Results are presented as the mean ± S.E.M. and tested for significance with the Wilcoxon test for paired differences or with the Student t-test.

3. Results and discussion

The first peak of insulin release in response to tolbutamide is known to appear one to two min earlier than in response to glucose [1,2,4]. When tolbutamide (20 mg/100 ml) and glucose (300 mg/100 ml) were administered together, the immediate insulin

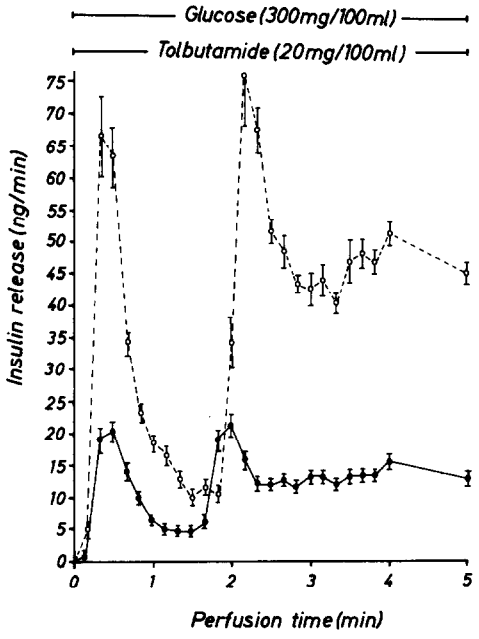

Fig.1. Effect of a combination of tolbutamide and glucose (1–5 min) on the immediate insulin secretory response of the perfused pancreas from fasted (●–●–●) and fed (○–○–○) rats. Mean ± S.E.M., Number of experiments: N = 10 (●–●–●) and N = 5 (○–○–○).

secretory response of the isolated perfused rat pancreas obtained from fasted animals consisted of two separate peaks (fig.1.). The first of these peaks appeared 98 ± 2 sec earlier ($2p < 0{,}01$) than the second one. The first of these peaks corresponds to the tolbutamide induced peak, the second one to the glucose induced peak.

The same insulin secretory pattern was obtained after perfusion of the isolated perfused pancreas from fed rats with tolbutamide plus glucose (fig.1). But the total amount of insulin secreted from the pancreas is more than three times higher ($2p < 0{,}01$) as reported previously for the perfused pancreas by Bosboom et al. [7]. These results emphasize the great importance of frequent early samplings in evaluating the immediate insulin secretory response to various stimuli. Other investigators were unable to demonstrate an immediate insulin secretory response consisting of two separate peaks because greater sampling intervals were chosen [2,3]. On the other side the results presented here confirm the finding of Curry that the total amount of insulin released from the pancreas in response to tolbutamide plus glucose is not higher than in response to glucose alone [2]. In our hands the total amount of insulin released from the pancreas during the first five minutes of secretion was even slightly but not significantly higher in response to glucose alone (74 ± 18 ng versus 62 ± 7 ng). This indicates that both glucose and tolbutamide induce insulin release from the same limited rapidly available pool as proposed already by Curry [2]. This may explain why glucose and tolbutamide release less insulin from the pancreas when administered simultaneously than after separate administration of each secretagogue.

The immediate insulin secretory response to tolbutamide appears about 90–100 sec earlier than in response to glucose. Bearing in mind that the dead space of our perfusion system is about 2 ml the stimulating agents need about 30 sec to reach the pancreas. On the basis of this calculation it can be said that tolbutamide exhibits its insulin releasing action as soon as it reaches the beta cell as shown by Bennett et al. [8]. On the other side there is a latent period of about 90–100 sec for glucose to induce insulin release. This indicates that the mechanisms of the isulin secretory action of tolbutamide and glucose are apparently different. It is tempting to speculate that tolbutamide may bypass some of the events originated by glucose.

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