Differences in Insulin Release in Response to Glucose and Tolbutamide Stimulation[1] (37607)

LESLIE L. BENNETT, DONALD L. CURRY, AND KITTY CURRY

The Hormone Research Laboratory and the Department of Physiology, University of California School of Medicine, San Francisco, California 94122; and The Department of Physiological Sciences, School of Veterinary Medicine, The University of California, Davis, California 95616

Previously published data in which glucose or tolbutamide were used to stimulate the isolated perfused rat pancreas indicate that release of insulin occurs more promptly following a stimulus of tolbutamide than it does to glucose. However, these experiments were not entirely satisfactory for determining the rapidity of the beta cell response following the two stimuli since the time resolution was never less than 30 sec/collection period (1). In addition, it has been shown that a glucose stimulation of more than 60 sec duration is necessary to produce a significant secretory response by the isolated perfused pancreas (2). In this report we have reinvestigated the pancreatic response to a glucose stimulus and to a tolbutamide stimulus using shorter collection periods of 15 sec. We also have determined the duration of a glucose stimulus necessary to produce a secretory response by keeping the glucose concentration constant (conditions not previously used) and administering the glucose within 1 cm of the arterial cannula inserted into the coeliac axis to minimize volume dilution. The duration of a tolbutamide stimulus necessary to produce insulin secretion has also been investigated. To the best of our knowledge data of this type have not heretofore been reported.

Materials and Methods. The isolated perfused preparation and the perfusing medium has been previously described (1). All pancreas donors were male rats of the Sprague–Dawley strain which weighed approximately 300 g. In all experiments flow rates were maintained constant at 10 cc/min, a flow previously shown to produce near maximum oxygen consumption (1). Insulin was assayed by the immunochemical method of Grodsky and Forsham (3) and total insulin secretion was calculated from the concentrations of insulin in the venous effluent and its volume.

Two types of experiments were performed. In the first type tolbutamide or glucose was delivered by a constant infusion pump as a square wave pulse of 2 min duration. These were infused in front of the warming coil on the arterial side of the preparation. The amounts of tolbutamide or glucose injected were calculated to produce stimulating concentrations of approximately three times threshold, that is, 12 mg/100 ml for tolbutamide and 210 mg/100 ml for glucose. In these experiments the total venous effluent was collected during each 15 sec interval to allow better time resolution for observing the secretory response.

In the second type of experiment tolbutamide or glucose was delivered for different periods of time just in front of the arterial cannula in the coeliac axis thus minimizing dead space on the arterial side of the preparation. The amounts of glucose used were calculated to produce maximal stimulating concentrations of 300 mg/100 ml, and the amounts of tolbutamide used produced a concentration of 20 mg/100 ml. A constant infusion pump was again used to deliver each agent. Following glucose infusion (either 30, 60 or 120 sec in duration), the total venous effluent was collected during each 30 sec interval. When tolbutamide was administered, the total venous effluent was collected each

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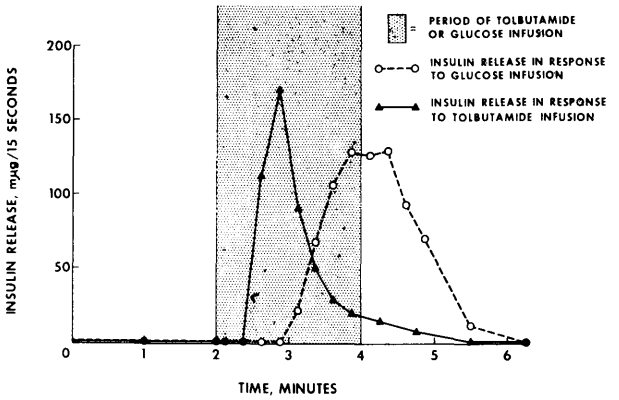

FIG. 1. Time course of insulin release following glucose (210 mg/100 ml) or tolbutamide (12 mg/100 ml) stimulations. Each curve represents the average insulin release by 5 different pancreas preparations in response to the stimuli indicated.

15 sec interval following beginning of stimulation, and the durations of stimuli used were either 1 sec, 3 sec, 5 sec, or 15 sec.

Results and Discussion. The data from the first type of experiment, that is, the 2 min stimulation by either tolbutamide or glucose, are shown in Fig. 1. The peak insulin secretion following tolbutamide stimulation occurs prior to any detectable release of insulin in response to glucose stimulation. The data also show that the peak rate of insulin release following tolbutamide stimulation occurs one full minute in advance of the maximum rate of insulin secretion following glucose stimulation. The shaded area in Fig. 1 represents the time during which the stimulating materials were being delivered from a constant infusion pump into the arterial supply of the isolated organ system. By analysis of the venous effluent we determined that neither tolbutamide nor glucose appeared until the collection period between 30–45 sec after the beginning of the infusion. This time delay represents the transit time of the perfusate through the tubing of the apparatus and the vascular system of the preparation. There is no release of insulin from the glucose stimulated pancreases during the 30–45 sec period following the onset of infusion, however, there is near maximal release of insulin from the tolbutamide-stimulated pancreases during this same time period. Also worthy of note is the fact that the decline in rate of insulin release following tolbutamide stimulation occurs extremely promptly even during maintained delivery of the tolbutamide into the arterial supply. The data from individual experiments show that the secretion rate of each tolbutamide stimulated pancreas had fallen to less than half its maximum rate 45 sec before the secretion rate of any glucose stimulated pancreas had reached its maximum.

The data from the experiments in which variable durations of glucose stimulations were used are shown in Fig. 2. These data show that following a 30-sec glucose stimulus, 3 pancreases showed no response and the other only a very slight response. For the 60-sec stimulation, all 4 preparations exhibited significant responses although the magnitude was not great. With a 120-sec stimulation not only did all 4 preparations exhibit a significant secretory response but the magnitude and duration was much greater than that which occurred following either of the two shorter periods of stimulation.

In Fig. 3 are shown the results from the experiment in which tolbutamide infusions of variable duration were used. Most striking is the fact that 4 of 5 preparations released significant amounts of insulin in response to a tolbutamide delivery of 1 sec duration. With stimuli of longer durations (3, 5, and 15 sec) each of 4 preparations responded by releasing significant amounts of insulin and there is a trend for more insulin to be released in response to the longer durations of stimulation. In each case the beginning of the tolbutamide injection was at the end

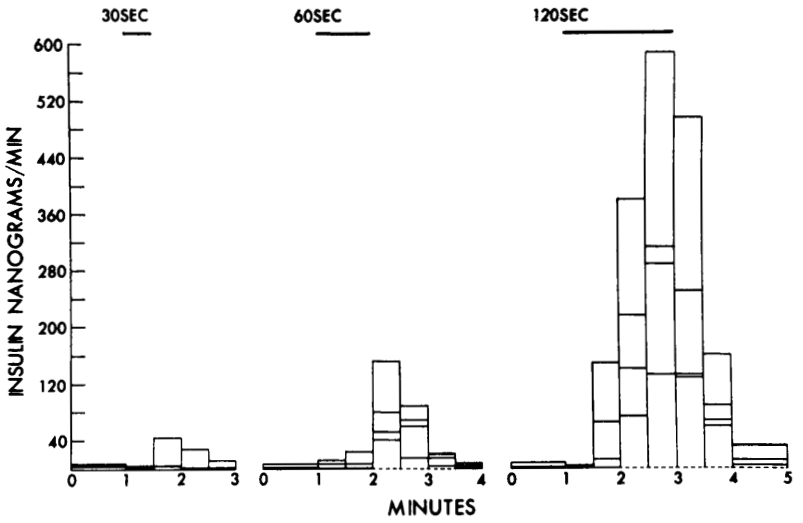

FIG. 2. Secretory response in 12 different experiments where the duration of glucose stimulation was 30 sec, 60 sec, or 120 sec. The width of the column indicates the duration of the collection period. Individual lines parallel to the X axis indicate by their height the secretion rate of insulin in ng/min. There were 4 different preparations for each of the time intervals. When the secretory responses were identical or so close together that individual lines could not differentiate them, the number of responses is indicated by the thickness of the horizontal line.

of the 1st min of collection. In no case was there a significant increase in the insulin level in the venous effluent collected in the first 15 sec after beginning of injection, but in all cases where secretion occurred it was present in the second 15-sec collection period. Using Evans blue dye as a plasma marker we determined that following a one sec injection, dye appeared in the first 15-sec collection tube only after approximately 8 sec

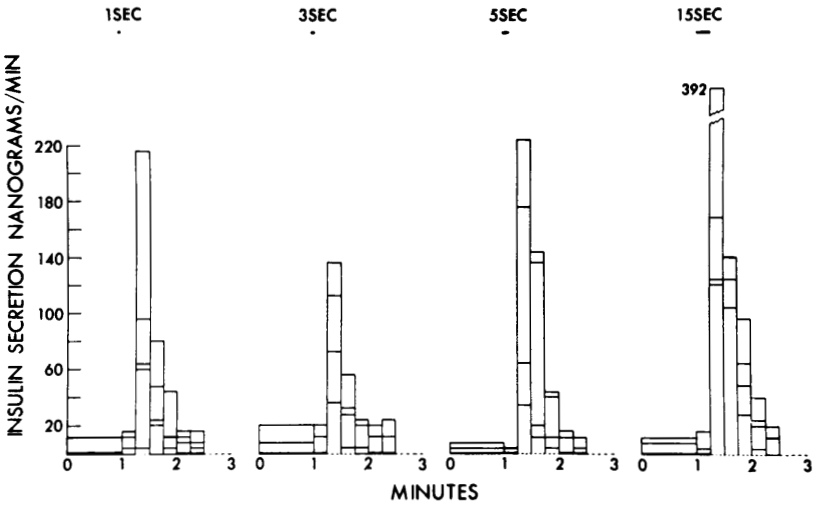

FIG. 3. Secretory response of 17 different experiments to 4 different durations of tolbutamide stimulation. The width of the column indicates the duration of the collection period. The height of the line parallel to the X axis indicates the secretion rate in ng/min. Where secretion rates were identical or so close together that individual lines could not differentiate them, the number of responses is indicated by the thickness of the line. There were 5 experiments at the 1-sec duration and for each of the other 3 time durations there were 4 experiments.

of the collection period had elapsed. However, 65%–70% of the total dye was in this first 15-sec collection tube. Approximately 97% of the dye traversed the preparation and the collecting tubing by the end of the first 30 sec following injections. These facts lead to the estimate that there is a period of 8–15 sec between the presentation of tolbutamide in the capillaries of the Islets of Langerhans and the appearance of insulin in the same capillaries. This time must be that necessary for the tolbutamide to diffuse across the capillary wall, through the interstitial spaces to the beta cells, cause release of insulin, and for insulin to diffuse back through the interstitial spaces and across the capillary wall into the capillary blood. The fact that tolbutamide was injected for only 1 sec cannot be interpreted as meaning that it was presented to the beta cells in the pancreas for only 1 sec. There was inevitably some dilution in the circulating perfusate, thus prolonging its presentation to the cells. Similarly the fact that it took approximately 22 sec for 97% of the plasma marker (Evans blue dye) to traverse the preparation and appear in the venous effluent, cannot be used as evidence that this was the maximum time during which tolbutamide was being presented to the beta cells. This is due to the fact that the preparation contains, in addition to the pancreas, other organs (spleen, stomach, omentum, and the proximal portion of the duodenum) through which both the tolbutamide and the plasma marker also would have traversed. It seems reasonable to assume that the agents traversed the pancreas in a much shorter period than the 22 sec total lapsed time, however, it is impossible to estimate how short this duration may have been.

Currently, there is much conjecture that glucose may have a dual function in stimulating insulin release. Some metabolic product of glucose may be required for insulin secretion and in addition there may be a gluco-receptor on the beta cell membrane which may be activated during glucose-stimulated insulin release (4). One of us has reported elsewhere (5) that other agents, such as mannose, may be capable of stimulating such a receptor. Conversely, it has long been known that tolbutamine is capable of stimulating insulin secretion, even in the absence of glucose (2). Whether tolbutamide activates the same receptor site(s), or even the same metabolic pathway, as does glucose, has yet to be established. However, the results reported in this paper showing the tremendously shorter activation time required for tolbutamide-stimulated insulin release, as opposed to glucose-stimulated secretion, would lend credence to the possibility that tolbutamide may act at a receptor site independently of glucose, bypassing the metabolic insulin secretory pathway which is activated by glucose.

Summary. With stimuli lasting a total of 2 min duration the peak insulin release from the isolated perfused rat pancreas occurs approximately 1 min earlier after a tolbutamide stimulus than a glucose stimulus. A one sec tolbutamide stimulus is adequate to produce significant insulin secretion but a glucose stimulus must be in excess of 30 sec to produce significant secretion.

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