A DIFFERENT PATTERN OF INSULIN RELEASE IN THE PERIFUSED ISLETS OF THE RABBIT

O. García Hermida and J. Gómez-Acebo
Laboratorio de Morfología. Instituto Gregorio Marañón
Centro de Investigaciones Biológicas. C.S.I.C.
Velázquez 144, Madrid 6. Spain

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SUMMARY

The rôle of the microtubular-microfilament system in the glucose mediated insulin secretory response has been investigated though a functional comparative study of perifused endocrine islets isolated from the pancreases of rats and rabbits. While microtubules are the main contractile organelles in the rat islets, microfilaments predominate in the beta cells of the rabbit. Rat islets displayed a biphasic response to glucose stimulated insulin secretion while in the rabbit the response was characterized by a multiphasic pattern. The short duration phases of glucose mediated insulin secretion in the rabbit resemble the "first phase" shown in the rat islets. On the basis of these findings it is suggested that the microtubules may be mainly involved with the mechanisms of sustained insulin released during glucose stimulation.

INTRODUCTION

It has been suggested that the microtubular-microfilament system was involved in the intracellular transport of beta granules following glucose stimulation of insulin secretion (1,2,3). The pattern of insulin secretion has been shown to be biphasic in man (4,5) as well as in isolated perfused rat pancreas or perifused preparations of isolated rat islets (6,7). At present there is no agreement on whether or not both phases of insulin release occur under the same secretory and control mechanisms. In a recent report the morphological relationships between the beta cell secretory granules and the microtubular-microfilament system were described during glucose mediated insulin release in vitro (8). It was suggested that the microtubules could act as channels through

which insulin molecules could be directed to the cell surface. On the basis of that work it was also postulated that the microtubular system would be preferentially involved in the second phase of insulin release. In order to test this hypothesis it was found interesting to carry out a comparative study of the glucose mediated insulin release in animal species which microtubular-microfilament system composition were qualitatively different.

The experiments herein reported will show that the rabbit, which beta cells contain predominantly microfilaments in their cytoplasm (9,10) and few microtubules (14), display a different functional response than the rat beta cell in which microtubules are the main contractile organelles (8).

MATERIAL AND METHODS

Islets were isolated by the collagenase technic (3,11) from male albino rats weighing 150 to 200 grs. or from male rabbits weighing 1.200 to 1.500 Kgrs. A minimum of one hundred islets per experiment were perifused following a procedure recently described by Lacy and col. (3,11). Originally perifusions were done using a bicarbonate buffer containing 5 mg/ml of bovine albumin (fraction V, Sigma Chemical Co., St. Louis). With this medium the rate of insulin release obtained with the isolated islets of the rabbit was very low. Hence after numerous trials and errors in many experiments 2 mg/ml of gelatine (Riedel-De Haën AG Seelze, Hannover) was used instead of the bovine albumin. Glucose (D+-glucose monohydrat, Merck, Darmstadt) 0.6 mg/ml or 3 mg/ml was employed as indicated. The perifusion fluid was collected in graduated tubes at either one minute or five minute intervals, the volumes were recorded and 0.5 ml aliquots were removed for insulin assay. A double chamber technic was used in some of the studies. In these experiments the islets of one rabbit or of two rats combined were placed in each chamber. The chambers were perifused

simultaneously with the same peristaltic pump. Islets in one of the chambers were perifused with the albumin containing buffer, those in the other chamber with the gelatine containing buffer. The insulin content of the perifusate was measured by the inmuno-assay technic of Wright and col. (12). Crystalline porcine insulin (Lilly) was used as the standard and porcine insulin labelled with I^{125} with a specific activity of 5-11 mC/mg was comercially obtained (Sorin, Vercelli). The anti-insulin serum used in the assays was produced in guinea pigs inmunized with crystalline porcine insulin and was kindly supplied by Dr. P.H. Wright (Indiana University, Indianapolis). The rate of insulin secretion was expresed as μUnits of insulin x islet^{-1} x minute^{-1}. At least six experiments were performed for each experimental situation. A type experiment has been graphycally represented.

RESULTS

Figure 1 shows the biphasic response of rat perifused islets after glucose (3 mg/ml) stimulation. With the perifusion system employed in these studies, the maximum rate of secretion in the first phase ocurred four to five minutes after changing from the low glucose (0.6 mg/ml) to the high glucose medium (3mg/ml) and lasted between three to five minutes (Fig. 1). The rate of secretion in the second phase reached a plateau about twenty minutes after changing the medium and remained at a relatively constant level (Fig. 1). There was no difference in the rate of insulin secreted during the incubation procedure when the medium contained albumin or gelatine (Fig. 1 AB).

The response of rabbit islets following sustained glucose stimulation (3 mg/ml) was different in several respects from the response of rat islets (Fig. 2). The maximum rate of secretion in the "first phase" occurred four to five minutes after changing from the low glucose (0.6 mg/ml) to the high glucose medium (3 mg/ml) and lasted between three to five minutes (Fig. 2B) after that,

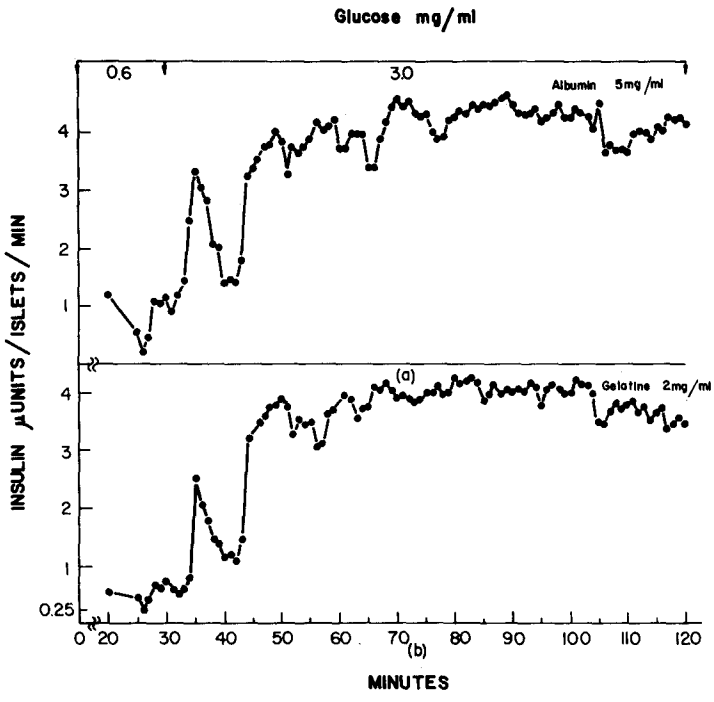

Figure 1. Biphasic pattern of insulin secretion following stimulation of perifused rat islets with glucose (3 mg/ml). A) Perifusion media with albumin. B) Perifusion media with gelatine.

there was no "second phase" of sustained insulin release as shown in the rat. However after four or five minutes there was a new peak of insulin release of the same characteristics as the first one described (Fig. 2B). From there on these peaks showed up at intervals, which frequency was higher in the first fifty to sixty minutes of glucose stimulation and more spaced after that period (Fig. 2B). Usually the peaks showed a duration between three to five minutes and there was no consistent frequency in their appearance. There were marked differences in the rates of insulin secreted by the rabbit islets in regard to the presence of albumin or gelatine in the medium (Fig. 2 AB). Whether albumin was present there was a very low insulin output. In the presence of gelatine the rate of insulin secretion was enhaced reaching approximately

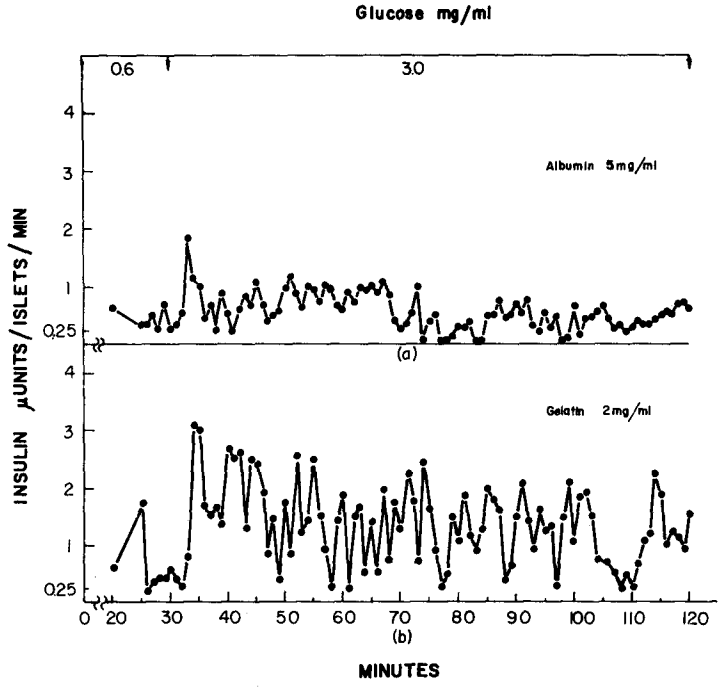

Figure 2. Insulin secretion following stimulation of perifused rabbit islets with glucose (3 mg/ml). A) Perifusion media with albumin. B) Perifusion media with gelatine.

twenty per cent of the rates obtained with the perifused rat islets.

DISCUSSION

From the above results it is clear that there was a significant difference in the patterns of insulin release obtained on perifusion of rat and rabbit islets respectively in presence of 300 mg per cent glucose. The insulin secretion in the rat was clearly biphasic, just as it has been previously described, the second phase being characterized by a sustained insulin release. The rabbit islets displayed an insulin secretory response characterized by a "spiky" pattern. This short phases of insulin release in the rabbit islets resemble those shown by the rat during the "first phase" of insulin secretion. This response may indicate a lack of "second phase" or sustained insulin release as shown in the rat.

This difference between patterns of insulin release by rat and rabbit islets are at present difficult to explain but of main importance could be the differences in ultrastructural organization of the contractile organelles in the beta cells of these two species. In general terms the rat beta cells contain numerous microtubules and few microfilaments in their cell webs (8). The rabbit beta cells contain numerous microfilaments and few microtubules (14). In recent works two different mechanisms have been suggested for the rôle of the microtubule in insulin release in the rat. The first one (1,2) was that the mature beta secretory granules become attached to the microtubular system, glucose metabolism triggering a change in the physical conformation of the microtubules, thus resulting in the granules being displaced to the cell surface for being released in tandem by emiocytosis. This mechanism has been postulated to be the only one operative in the two phases of insulin release (1,2). In a recent work (8) an alternate mechanism for the rôle of the microtubules during the second phase of insulin release was suggested, fusion of the beta secretory granules with the microtubules, discharge of secretory contents into them and transportation of these content through the microtubules to the plasma membranes for discharge. This scheme also potulates the existence of two different mechanisms of insulin release one for each one of the first and second phases, the microtubule being most important during the second phase. The first phase would be mainly determined by emiocytosis of beta secretory granules. The herein reported findings of lack of glucose mediated sustained insulin release in the rabbit which beta cells contain few microtubules seem to support the idea of the principal rôle of this organelles in the "second phase" of insulin secretion. On the other hand due to the abundance of microfilaments in this cells it is tempting to atribute to this organelles their participation in the appearance of short pulses of insulin secretion as in the so called "first phase" in the rat (Fig. 1A). The fact that comercial bovine albumin (fraction V Cohn) prevents glucose mediated insulin

release in the rabbit islets and not in the rat islets, indicates some differences in the metabolism of both cells. The finding that the rate of insulin released into the medium by the rabbit islet is less than the one released by the rat islet is in accord with the response of these two species to the administration of anti-insulin serum (13). These findings seem to support the conclusion that rat secretes more insulin per islet than the rabbit, suggesting a different funcional reserve in both species.

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