DIGITOXIN, A MULTIPLE SPIKE STIMULATOR OF INSULIN RELEASE IN THE PERIFUSED ISLETS OF THE RAT.

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SUMMARY

On the basis of an analogy between stimulus-secretion coupling in the B-cell and excitation-contraction coupling in muscle, we have made the hypothesis that a cardiac glycoside Digitoxin (0.25 μM) by possible action on B-cell contractile filaments may produce insulin release. Our findings indicate that Digitoxin in the presence of 30 or 60 mg per cent glucose produces insulin release by repeated activation of the "first phase" with a pattern of secretion similar to what has been described recently to be the normal in the glucose perifused rabbit islets. This cardiac glycoside also produced a partial inhibition of glucose (300 mg per cent) induced insulin release, indicating a competitive action of these two substances. Digitoxin being the only drug reported up to the present that stimulating insulin release, partially inhibits glucose induced insulin release.

INTRODUCTION

In recent work the existence of two different mechanisms of insulin release one for each one of the first and second phases of that release has been postulated (1,2). The first phase would be mainly determined by emiocytosis of beta secretory granules in which the microfilaments would participate and the microtubules would have a predominant role during the second phase of the release. At present a two compartment model to account for the biphasic pattern of insulin release with glucose has been suggested (3). The esential feature of the model is the suggestion that only a small proportion (or compartment) of pancreatic insulin (2%) is available for rapid release and the remainder compartment is released at a much slower rate. Also, considering that extracellular calcium is

required for glucose or any other insulinotropic agent to stimulate secretion (4) and that an analogy between stimulus-secretion coupling in the B-cells and excitation-contraction coupling in muscle has been raised (5) we have made the hypothesis that a cardiac glycoside (Digitoxin) by posible acting on B-cell contractile proteins analogous to heart contractile proteins (microfilaments) may produce insulin release by repeated activation of the "first phase" with a release pattern similar to what has been described recently in the normal perifused rabbit islets (2).

MATERIAL AND METHODS

Islets were isolated by the collagenase technique (6) from male or female albino rats weighing 150 to 200 grs. A minimum of one hundred islets per experiment were perifused following a procedure recently described (7,2). The perifusion medium a bicarbonate buffer had the following ionic composition: Na^+ 139, K^+ 5, Mg^{++} 2, Ca^{++} 2, Cl^- 124 and CO_3H^- 24 mEqui./l; pH 7.4. Added to the media as required; Digitoxin 0.2 µgr/ml (0.25 µM) (Nativelle, France). The effect of Digitoxin was tested over different ranges of glucose (D+ glucose, Merck, Darmstadt), concentrations used were 30, 60 and 300 mg/100 ml. The perifusion fluid was collected in graduated tubes at one minute intervals, the volumes were recorded and 0.5 ml aliquots were removed for insulin assay. A double chamber technique was used in many of the studies. In these experiments the islets 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 buffer containing glucose (30, 60 or 300 mg/100 ml) and ethanol (0.71 mM), those in the other chamber with the perifusion media plus Digitoxin (0.25 µM). The insulin content of the perifusate was measured by the inmunoassay technique of Wright and col. (8). Crystalline porcine insulin (Lilly) was used as the standard and porcine insulin labelled with ^{125}I with a specific activity of 5 - 11

mC/mg was commercially 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 expressed as uU of insulin x $islet^{-1}$ x $minute^{-1}$. At least six experiments were performed for each experimental situation. A type experiment has been shown graphycally.

The islets of Langerhans before and after perifusion were fixed in 3% phosphate buffered gluteraldehyde for 1/2 hour at room temperature and post-fixed in 2% cold osmium tetroxide, dehydrated in ethanol and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate followed by lead citrate, mounted on uncoated grids and examined with a Phillips EM 30 or a Jeol 100-B.

RESULTS

Figure 1 shows the response of rat islets following sustained digitoxin stimulation (0.25 μM) in the presence of 30 or 60 mg glucose/100 ml. "First phase" of insulin secretion occurred four to five minutes after changing from the low glucose (0.3 mg/ml or 0.6 mg/ml) to the digitoxin containing medium and lasted between two to four minutes. After that there was no "second phase" of sustained insulin release as shown in the glucose (3 mg/ml) stimulated islets (figure 2) but several new peaks of insulin release of the same characteristics as the first one described. These peaks showed up at intervals with a duration of one to four minutes and there was no consistent frequency in their appearance. The total quantities of insulin released during digitoxin stimulation were 108 ± 3.92 μU/islet. Figure 2 shows the biphasic response of rat perifused islets after glucose (300 mg/100 ml) stimulation. The rate of secretion in the "first phase" lasted between three to five

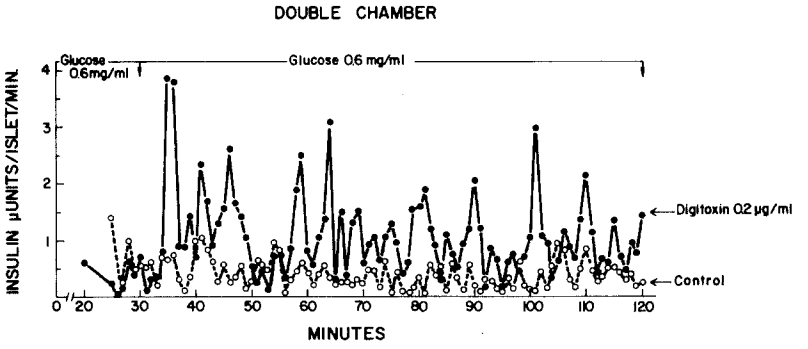

Figure 1.- Insulin secretion following stimulation of perifused rat islets with Digitoxin (0.25 µM) showing a multiple spike pattern of insulin release.

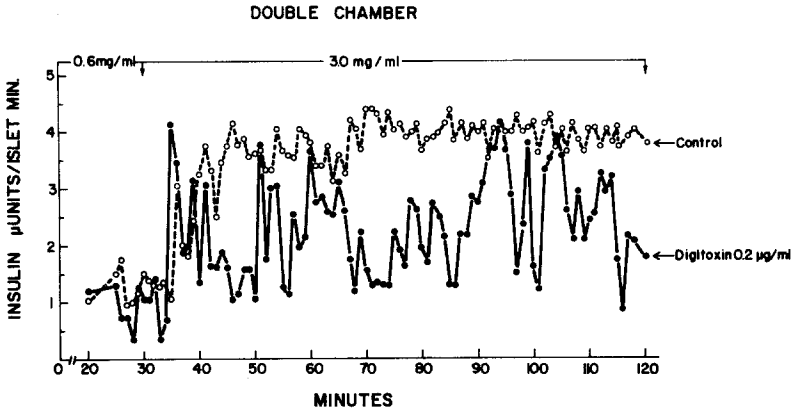

Figure 2.- Insulin secretion following stimulation of perifused rat islets with glucose (300 mg/100 ml) and with glucose (300 mg/100 ml) plus Digitoxin (0.25 µM).

minutes. The rate of secretion in the "second phase" reached a plateau about twenty minutes after changing the medium and remained at relatively constant level (figure 2). The total quantities of insulin released during this period were 313 ± 11.57 µU/islet. The response of rat perifused islets following sustained administration of glucose(300 mg/100 ml) plus digitoxin (0.25 µM) was different in several respects from the response obtained by administering glucose (300 mg/100 ml) alone.

The biphasic pattern of insulin release was maintained but the total quantities of insulin obtained (190 ± 41.33 μU/islet) were diminished in relation to the values obtained when the islets were stimulated with glucose (300 mg/100 ml). The "second phase" of this release was characterized by a low plateau of release interspeed with higher peaks (figure 2).

The digitoxin treated B-cells showed an excellent preservation of their structures. A report with the morphological findings will be published in the near future.

DISCUSSION

From the above results it is clear that digitoxin (0.25 μM) in the presence of 30 or 60 mg per cent glucose produced insulin release on perifusion of rat as expected. These islets, under the experimental conditions used, displayed an insulin secretory response characterized by a "spiky" pattern. These alternate phases of insulin release resemble those shown by the rat during the "first phase" of glucose (300 mg per cent) stimulated release and was similar to the pattern of insulin release obtained in the glucose stimulated perifused normal rabbit islets (2), these indicating that very likely there was a constant activation of the mechanisms of release for the "first phase". In view of the low concentrations used, the stimulant action of digitoxin is more likely to be due to its effect on Na^+ and K^+ active transport (9), this ionotrophic action increasing perhaps a labile calcium fraction in the B-cells and the binding of potassium and calcium ions to the contractile proteins (microfilaments) (10) rather than to any effect of the sugar molecule which is part of glycoside. The constant activation of the microfilament system which likely (1,2) regulates in some unknown way the "first phase" of insulin release, would produce the appearance of pulses of insulin secretion as in the so-called "first phase" explaining the "spiky"

pattern of this release. Nevertheless a very interesting finding was the fact that digitoxin (0.25 μM) partially inhibits glucose (300 mg/100 ml) stimulated insulin release, perhaps indicating a competitive action of these two substances at the level of their action mechanism and in this regard it is very interesting that digitoxin is the only drug reported until now that, stimulating insulin release per se, partially inhibits glucose stimulated insulin secretion. At present the characteristics of this competition is being investigated further in our laboratory, specially with the idea in mind that the competition may be at the level of a hypothetical glucoreceptor (11) in the B-cell (binding of the glycoside sugar molecule). All these considerations make digitoxin induced insulin release, an interesting model for the study of this release and also may have important implications in the possible farmacological actions of some cardiac glycoside in non-muscular cells and in the farmacological treatment of cardiac patients.

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