GLUCOSE-INDUCED FIRST PHASE INSULIN RELEASE IN THE ABSENCE OF EXTRACELLULAR Ca^{2+} IN RAT ISLETS

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1. Introduction

Islets of Langerhans respond to a rapid and constant increase of the glucose concentration with biphasic release of insulin, a pattern that is observed in the portal vein in humans [1] and in isolated pancreatic preparations [2–4]. The presence of extracellular Ca^{2+} has been thought essential for both phases of insulin release [2,5,6]. However, recent studies have suggested that the first phase is dependent upon the utilization of cellular calcium, whereas the second phase depends both on cellular calcium and increased uptake of Ca^{2+} from the extracellular fluid [3,7], Those experiments were performed using either inhibitors of Ca^{2+} uptake [3] or islets with increased calcium stores [7]. In islets with normal calcium stores, a preserved first phase insulin release in the absence of extracellular Ca^{2+} has not been demonstrated. This study was performed to examine the differential sensitivity of the two phases to Ca^{2+} deprivation. It was found that first phase insulin release could occur in the absence of extracellular Ca^{2+}, when Ca^{2+} was removed from the medium before the glucose-induced rise of insulin release.

2. Materials and methods

Collagenase isolated rat islets [8] were maintained for 46 h in tissue culture medium 199, containing 1.8 mM $CaCl_2$ and 8.3 mM glucose [9]. 40 islets/chamber were perifused as in [5,9]. The chamber volume was 70 μl and the flowrate 1.2 ml/min which results in a turnover of 17 chamber vol./min. The standard perifusion medium consisted of Krebs-Ringer bicarbonate buffer (KRB) containing 1.0 mM $CaCl_2$, 0.5% dialysed bovine serum albumin and glucose concentrations as indicated. At different time points the perifusate was changed to a KRB buffer prepared without the addition of $CaCl_2$ (Ca^{2+}-deprived medium). The total Ca^{2+} concentration of this medium was 14.0 ± 1.1 μM (n = 3) as determined by atomic absorption spectrophotometry. When Ca^{2+} was removed the measured Ca^{2+} concentration decreased from 1 mM to basal values (14.6 ± 2.0 μM, n = 5) in the sample collected during the second minute. Assuming an exponential decay curve for the decrease it can be calculated from the measured Ca^{2+} concentration over the first minute (63.8 ± 2.0 μM, n = 5) that the concentration surrounding the islets was <25 μM 15 s after the change. Insulin was determined by radioimmunoassay [10] using a rat standard. Insulin release values for every single chamber during first and second phase were calculated by integrating first 5 min of stimulation or the subsequent 39 min for first and second phase, respectively, after individual baseline subtraction. The sources of the materials have been described [3]. Data given as means ± SEM were analysed by Student's unpaired t-test.

3. Results

Glucose at 16.7 mM elicited a biphasic insulin release at 1 mM Ca^{2+} throughout (fig.1). One minute after high glucose had reached the islets, insulin release started to rise, a peak was reached at 2–3 min. A nadir at 5–7 min was followed by an increasing second phase. Total release above baseline during

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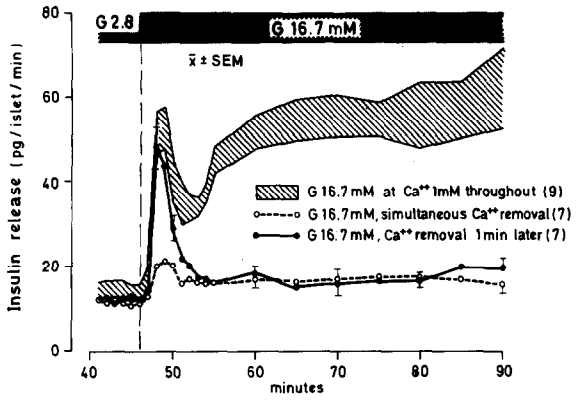

Fig.1. Effect of Ca^{2+} removal on glucose-induced insulin release. Islets were perifused for 46 min with a medium containing 2.8 mM glucose and 1 mM Ca^{2+} and then stimulated for 44 min with 16.7 mM glucose. Ca^{2+} deprived medium was imposed either simultaneously with 16.7 mM glucose or 1 min later as indicated. No. obs. in parenthesis. G, glucose.

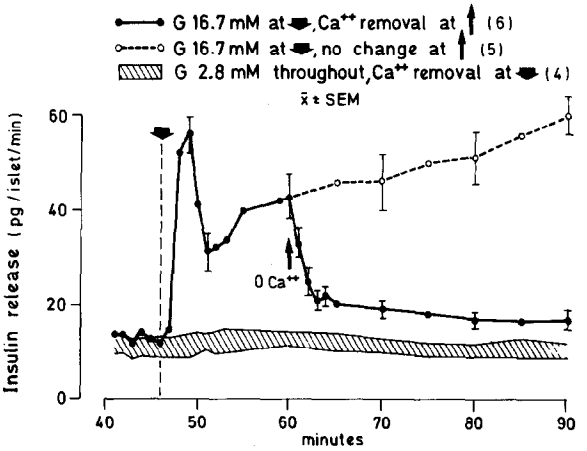

Fig.2. Effect of Ca^{2+} removal during second phase insulin release. Islets were perifused for 46 min with 2.8 mM glucose and 1 mM Ca^{2+}. One group was then stimulated for 44 min with 16.7 mM glucose (continuous line). At the 60th min the islets were exposed to Ca^{2+}-deprived medium. Islets not subjected to Ca^{2+} removal are represented by the interrupted line. Another group of islets was exposed to Ca^{2+}-deprived medium at 46 min in the continued presence of 2.8 mM glucose (shaded area). G, glucose.

first phase (5 min) was 117 ± 16 pg/islet and 1494 ± 177, $n \doteq 9$, during second phase. When islets were exposed to Ca^{2+} deprived medium (14 μM) simultaneously with the increase in the glucose concentration (1 min before the rise in insulin release) insulin release was markedly inhibited during both phases. Release was 31 ± 4 pg/islet during first phase and 225 ± 87 pg/islet ($n = 7$) during second phase, an inhibition of 74% and 85% when compared with the situation at 1 mM Ca^{2+} ($p < 0.001$ for both).

Next, Ca^{2+} was removed 1 min after the increase of the glucose concentration, immediately before a significant rise in insulin release. Insulin release increased markedly despite the simultaneous decrease of the Ca^{2+} concentration. Compared with islets perifused at normal Ca^{2+}, there were no significant differences in the rate of insulin release until the 51st minute, i.e., 5 min after glucose stimulation. Insulin release above baseline during first phase (101 ± 12 pg/islet, $n = 7$) was similar to that at 1 mM Ca^{2+} ($p > 0.40$). In contrast, second phase insulin release was markedly inhibited (240 ± 113 pg/islet) and was similar to Ca^{2+} removal at the time of glucose stimulation.

Two control experiments were performed (fig.2). To see whether Ca^{2+} removal per se could cause a transient stimulation of insulin release Ca^{2+} was removed in the continuous presence of 2.8 mM glucose. No change in insulin release occurred. To test the effect of Ca^{2+} deprivation during second phase Ca^{2+} was omitted after 60 min perifusion, i.e., 14 min after glucose stimulation. Insulin secretion decreased promptly in the first min after Ca^{2+} removal and reached a plateau level between 2 and 3 min with release rates above basal. Thus, both control experiments show that Ca^{2+} removal per se did not stimulate insulin release.

4. Discussion

An increased concentration of ionised Ca^{2+} in the β-cell cytosol is thought to mediate glucose-stimulated insulin release [3,4,11,12]. Such an increase could result from a change in the distribution of cellular calcium or from increased uptake of Ca^{2+} from the extracellular fluid or both. Although glucose enhances Ca^{2+} uptake during first phase insulin release [3,9,12] blockade of this stimulated uptake by verapamil did not affect the first phase, while second phase release was inhibited by 50% [3]. It was therefore concluded that the first phase is not dependent on increased Ca^{2+} uptake, but rather on cellular calcium. Furthermore, islets with increased cellular calcium stores perifused at low extracellular Ca^{2+} (0.1 mM) showed a well preserved first phase and a 50% inhibited second phase [7].

The failure of glucose to elicit insulin release after prolonged absence of Ca^{2+} [2,5,6] seems at variance with the above conclusion. Moreover, in the present study, both phases of insulin release were markedly reduced when Ca^{2+} was removed at the time of glucose stimulation. Ca^{2+} deprivation, however, has at least two effects on the islets. Influx of extracellular Ca^{2+} is minimized and cellular calcium is decreased [5,11,13]. This implies an imbalance of Ca^{2+} influx and efflux which combined with the high turnover of Ca^{2+} across the plasma membrane [3] could rapidly affect insulin release. For instance, a hypothetical labile calcium pool in close proximity to the β-cell membrane may be of critical importance for insulin release [6,11]. An increased amount of pyroantimonate precipitates (presumably containing calcium) was found at the inside of the plasma membrane 3 min after exposure to glucose [13]. This was not seen when Ca^{2+} was omitted at the time of glucose stimulation [13]. The marked inhibition of insulin release under similar conditions in the present study could be due to an inability of glucose to raise cytosolic Ca^{2+} either directly or indirectly because within 1 min depletion of a critical calcium pool occurs. This was further examined by introducing Ca^{2+} deprived medium 1 min after the start of glucose stimulation, i.e., before a significant rise of insulin release. Despite the rapid decrease of extracellular Ca^{2+} (see section 2) a marked increase of insulin release resulted and a normal first phase was seen.

Clear differences exist between the extra- and intracellular Ca^{2+} requirements of first and second phase as shown by the results of Ca^{2+} removal just discussed and those performed during second phase, in which only a rapid decrease of insulin release occurred (fig.2). This is in accord with conclusions that second phase insulin release is more dependent on extracellular Ca^{2+} than first phase [3,7].

In view of the apparent difference in the regulation of the two phases of insulin release it is of interest that in certain types of human diabetes, particularly the early insulin response to glucose is defective [14,15]. It is noteworthy that in the diabetic Chinese hamster a defect in cellular Ca^{2+} handling appears to be involved in the impaired insulin release [16] and that this defect seems to affect mainly the early insulin response [16,17].

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