RESTORATION OF GLUCOSE-INDUCED INSULIN RELEASE IN THE ABSENCE OF EXTRACELLULAR CALCIUM BY INHIBITION OF THE Na^+- PUMP

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1. Introduction

An increase in the concentration of ionized Ca^{2+} in the cytosol of the β-cell is thought to be a controlling factor in stimulus-secretion coupling of glucose-induced insulin release [1–4]. 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. Both in vivo [5] and in vitro [1–4] a constant increase of the glucose concentration results in a spike-like first phase and a progressively rising second phase of insulin release. Presence of extracellular Ca^{2+} has been thought necessary for both phases, since glucose does not stimulate insulin release after exposure to Ca^{2+}-deprived media [3,6]. Exposure to such media, however, does not only minimize the effects of extracellular Ca^{2+}; it also decreases cellular calcium content [3,6] which may make it impossible to distinguish between effects of intra- or extracellular Ca^{2+} [7]. Therefore we investigated whether glucose can utilize cellular calcium to cause insulin release in Ca^{2+} depleted medium. Since in other cells, the inward Na^+ gradient is involved in the extrusion of Ca^{2+} from the cell [8,9], conditions which decrease the Na^+ gradient were imposed. These were the application of ouabain and the removal of extracellular K^+ to inhibit Na/K ATPase [10,11]. While Ca^{2+} removal at the time of glucose stimulation markedly reduced insulin release, this reduction was completely overcome during first phase and partially during second phase if Na/K ATPase was inhibited when glucose was applied.

2. Materials and methods

Collagenase-isolated rat islets [1] were maintained for 2 days in tissue culture medium 199 at 8.3 mM glucose [1,6]. Thereafter, 40 islets per chamber were perifused as described [1,6]. The chamber volume was 70 μl and the flow rate 1.2 ml/min, the turnover was 17 chamber volumes per min. Standard perifusion buffer contained 1.0 mM $CaCl_2$. Ca^{2+}-deprived medium was prepared without the addition of $CaCl_2$. Its Ca^{2+} concentration was 14.0 ± 1.1 μM (n = 3) as determined by atomic absorption spectrophotometry. When the perifusate was changed to Ca^{2+}-deprived medium, 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 2nd min. 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 less than 25 μM 15 s after the change. Insulin was determined by radioimmunoassay [12] using a rat standard. The sources of the materials have been described [1,6] except for ouabain (Merck, Darmstadt, FRG). Data are given as means ± SEM and were analysed by Student's unpaired t-test.

3. Results

At 1 mM Ca^{2+}, 16.7 mM glucose elicited a biphasic insulin release (fig.1). One minute after high glucose had reached the islets, insulin release started to rise to a peak which was reached at 2–3 min. A nadir at 5–7 min was followed by an increasing second phase. 0.1 mM ouabain added at the time of glucose stimulation had no significant effect on insulin release at either low or high glucose. This finding is similar to that described by others for the same concentration of ouabain [13]. When Ca^{2+} was removed simultaneously with glucose stimulation, insulin release was

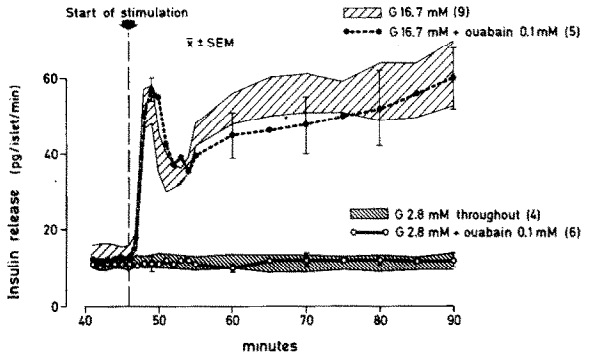

Fig.1. Effect of 0.1 mM ouabain on basal and glucose-induced insulin release. Following 2 days in tissue culture, islets were perifused with a Krebs-Ringer bicarbonate buffer containing 1 mM Ca^{2+}, 0.5% bovine serum albumin and 2.8 mM glucose [1]. After 46 min two groups of islets were stimulated for 44 min with 16.7 mM glucose. 0.1 mM ouabain was added after 46 min to buffer containing either 2.8 or 16.7 mM glucose. Numbers of observations in parentheses. G, glucose. Values are presented as mean ± SEM.

markedly inhibited during both phases (fig.2). Addition of 0.1 mM ouabain together with high glucose at the time of Ca^{2+} removal, greatly enhanced insulin release over both phases. During first phase (first 5 min) the inhibitory effect of Ca^{2+} removal was completely overcome whole second phase was restored to 43% of release at 1 mM Ca^{2+} (taking integrated insulin release above baseline). Ca^{2+} removal at 2.8 mM glucose throughout did not affect insulin release (fig.2). To exclude that the small amount of extracellular Ca^{2+} present could have contributed to the restoration of insulin release of ouabain, six experiments were performed with the addition of 0.1 mM EGTA to the Ca^{2+}-deprived medium. This did not significantly affect the restoration of glucose-stimulated insulin release by ouabain (results not included).

To see whether these marked effects of ouabain in the absence of extracellular Ca^{2+} were indeed due to its known inhibitory effect on the Na-pump, another condition which inhibits this pump was chosen, namely exposure to a medium prepared without K^+ (fig.3). K^+-deprivation at the time of glucose stimulation and Ca^{2+} removal gave similar results as those described for 0.1 mM ouabain. The inhibition of insulin release in the absence of Ca^{2+} was completely overcome during first phase; second phase was enhanced to 36% of insulin release at normal Ca (integrated insulin above baseline).

4. Discussion

The presence of a Na^+ pump in islets has been inferred from studies on ionic fluxes [14,15] and electrophysiological findings [16]. In addition, an enzyme with Na/K ATPase activity was found in rat islet homogenates [10,11]. Ouabain, in a dose similar to the one used here, inhibited this enzyme [10,11].

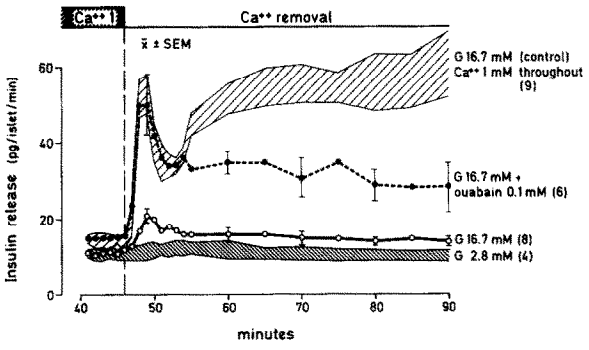

Fig.2. Effect of 0.1 mM ouabain on glucose-induced insulin release in Ca^{2+} deprived medium. Islets were perifused for 46 min with 2.8 mM glucose and 1 mM Ca^{2+} and then stimulated for 44 min with 16.7 mM glucose. When islets were exposed to Ca^{2+} deprived medium (14 μM) with or without 0.1 mM ouabain, this was applied after 46 min. A control group only subjected to Ca^{2+} removal after 46 min is included (lower shaded area). G, glucose.

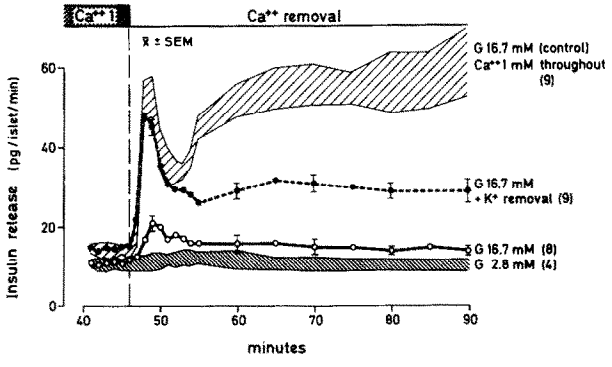

Fig.3. Effect of K^+ removal on glucose-induced insulin release in Ca^{2+} deprived medium. Islets were perifused for 46 min with 2.8 mM glucose and 1 mM Ca^{2+} and then stimulated for 44 min with 16.7 mM glucose. When islets were exposed to Ca^{2+} deprived medium, with or without K^+ removal, this was applied after 46 min. A control group only subjected to Ca^{2+} removal after 46 min is included (lower shaded area). K^+-deprived medium was prepared by replacing KCL and KH_2PO_4 by the equivalent sodium salt. G, glucose.

With respect to insulin release, ouabain at high glucose concentrations was generally not found to enhance insulin release [13,17]. At low glucose, varying results have been obtained depending on the pancreatic preparation and the dose employed ([13,17,18], and refs. cited in [17]).

The failure of ouabain to alter glucose-stimulated insulin release at normal Ca^{2+} contrasts markedly with its action in Ca^{2+}-deprived media. In seeking an explanation for this finding the effects of glucose on Ca^{2+} handling by islets have to be considered. Apart from stimulating Ca^{2+} uptake [1–4], glucose has been shown to inhibit Ca^{2+} efflux [2,6] and both mechanisms could increase cytosol Ca^{2+}. It is likely that a Na/Ca exchange takes place at the plasma membrane [1,18–20] like in other tissues [8,9]. Thus, the inward Na^+ gradient, which is maintained by the Na^+ pump, permits the extrusion of Ca^{2+} against its inwardly favorable electrochemical gradient. There is evidence that glucose exerts its inhibitory effect on Ca^{2+} efflux by interfering with Na/Ca exchange [19,20]. This action of glucose is not likely to occur via the Na-pump, since glucose and ouabain have opposite effects on Na^+ and K^+ handling in islets [14,15].

When the Ca^{2+} gradient is decreased by removal of extracellular Ca^{2+} (fig.2), the balance between the Na^+ and the Ca^{2+} gradient is disturbed. Since the Na^+ content, and thus most likely the Na^+ gradient, remains unaltered by Ca^{2+} removal [14], an increase in Ca^{2+} efflux should be expected. This has been demonstrated for $^{45}Ca^{2+}$ efflux from preloaded islets [21]. Likewise, in the islet perifusion system used here, Ca^{2+} removal also caused a rapid increase of $^{45}Ca^{2+}$ efflux from preloaded islets with a peak in the first min (results not shown). Therefore, Ca^{2+} removal will counteract the inhibitory effect of glucose on Ca^{2+} efflux (which would normally contribute to increase cytosol Ca^{2+}). When the Na^+ pump is inhibited by ouabain or K^+ omission, the Na^+ gradient is reduced [14] and therefore the driving force for the coupled Ca^{2+} efflux. A decrease of the sodium gradient has been shown to increase the ^{45}calcium content in isolated islets [18,22] which can be attributed to inhibition of Ca^{2+} efflux, since Ca^{2+} influx rates were not changed [22]. The expected inhibitory effect of ouabain on Ca^{2+} efflux [20] may act synergistically with that due to glucose and outweigh the impact of Ca^{2+} removal. This could, therefore, explain the restoration of insulin release (figs.2 and 3), if ouabain has a rapid onset of action. An onset of less than 1 min has indeed been shown in electrophysiological experiments for the depolarizing effect of both ouabain and K^+ removal [16]. Thus, the presence of ouabain could allow glucose to raise cytosol Ca^{2+} high enough by the use of cellular calcium to account for a normal first phase of insulin release in spite of the absence of extracellular Ca^{2+}. An additional effect of increased intracellular Na^+ could be the mobilization of stored calcium [18].

The finding that first phase is restored fully and second phase only partially is similar to results obtained when verapamil was used to block Ca^{2+} uptake via the voltage-dependent Ca^{2+} channel [1]. 5 μM verapamil, which totally blocked glucose-stimulated Ca^{2+} uptake over the first phase, had no effect on insulin release during this period, but second phase was inhibited by 55%. Comparable findings were obtained from islets with increased calcium stored perifused at low extracellular Ca^{2+} [23].

The results presented here demonstrate that extracellular calcium is not an absolute requirement for the stimulation of insulin release. Glucose appears to use cellular Ca^{2+} to generate first phase [24], whereas both a cellular source of Ca^{2+} and increased influx of Ca^{2+} from the extracellular fluid contribute to the full development of second phase. Derangements in islet Ca^{2+} handling appear to play a role in the defective glucose-stimulated insulin release of two diabetic animal syndromes [25,26] and in particular the mechanism(s) by which glucose utilises cellular calcium.

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References

[1] Wollheim, C. B., Kikuchi, M., Renold, A. E. and Sharp, G. W. G. (1978) J. Clin. Invest. 62, 451–458.
[2] Malaisse, W. J., Herchuelz, A., Devis, G., Somers, G., Boschero, A. C., Hutton, J. C., Kawazu, S., Sener, A., Atwater, I. J., Duncan, G., Ribalet, B. and Rojas, E. (1978) Ann. NY Acad. Sci. 307, 562–582.

[3] Hellman, B., Andersson, T., Berggren, P.-O., Flatt, P., Gylfe, E. and Kohnert, K.-D. (1979) Horm. Cell Regul. 3, 69–97.
[4] Siegel, E. G., Wollheim, C. B., Kikuchi, M., Renold, A. E. and Sharp, G. W. G. (1980) J. Clin. Invest. 65, 233–241.
[5] Elahi, D., Andersen, D., Brown, J. C., Debas, H. T., Hershcopf, R. J., Raizes, G. S., Tobin, J. D. and Andres, R. (1979) Am. J. Physiol. (1979) 237(2), E185–E191.
[6] Kikuchi, M., Wollheim, C. B., Cuendet, G. S., Renold, A. E. and Sharp, G. W. G. (1978) Endocrinology 102, 1339–1349.
[7] Borle, A. (1978) Ann. NY Acad. Sci. 307, 431–432.
[8] Blaustein, M. P. and Russell, J. M. (1975) J. Membr. Biol. 22, 285–312.
[9] Van Breemen, C., Aaronson, P. and Loutzenhiser, R. (1979) Pharmacol. Rev. 30(2), 167–208.
[10] Levine, S. R., Kasson, B. G. and Driessen, J. F. (1978) J. Clin. Invest. 62, 692–701.
[11] Kemmler, W. and Löffler, G. (1977) Diabetologia 13, 235–238.
[12] Herbert, V., Lau, K.-S., Gottlieb, C. W. and Bleicher, S. J. (1965) J. Clin. Endocr. 25, 1375–1384.
[13] Lambert, A. E., Henquin, J. C. and Malvaux, P. (1974) Horm. Metab. Res. 6, 470–475.
[14] Kawazu, S., Boschero, A. C., Delcroix, C. and Malaisse, W. J. (1978) Pfluegers Arch. 375, 197–206.
[15] Boschero, A. C., Kawazu, S., Duncan, G. and Malaisse, W. J. (1977) FEBS Lett. 83, 151–154.
[16] Meissner, H. P. (1976) J. Physiol. (Paris) 72, 769–786.
[17] Pace, C. S. (1979) Am. J. Physiol. 237(2), E130–E135.
[18] Donatsch, P., Lowe, D. A., Richardson, B. P. and Taylor, P. (1977) J. Physiol. (London) 267, 357–376.
[19] Herchuelz, A. and Malaisse, W. J. (1979) Diabetes 28, 107.
[20] Siegel, E. G., Wollheim, C. B., Renold, A. E. and Sharp, G. W. G. (1980) J. Clin. Invest. (in press).
[21] Malaisse, W. J., Hutton, J. C., Sener, A., Levy, J., Herchuelz, A., Devis, G. and Somers, G. (1978) J. Membr. Biol. 38, 193–208.
[22] Hellman, B., Sehlin, J. and Täljedal, J.-B. (1978) Pfluegers Arch. 378, 93–97.
[23] Kikuchi, M., Wollheim, C. B., Siegel, E. G., Renold, A. E. and Sharp, G. W. G. (1979) Endocrinology 105, 1013–1019.
[24] Siegel, E. G., Wollheim, C. B. and Sharp, G. W. G. (1980) FEBS Lett. 109, 213–215.
[25] Siegel, E. G., Wollheim, C. B., Sharp, G. W. G., Herberg, L. and Renold, A. E. (1979) Biochem. J. 180, 233–236.
[26] Siegel, E. G., Wollheim, C. B., Sharp, G. W. G., Herberg, L. and Renold, A. E. Am. J. Physiol. (in press).