STIMULATION OF INSULIN RELEASE AFTER RAISING EXTRACELLULAR CALCIUM

Bo HELLMAN
Department of Histology, University of Umeå, S-901 87 Umeå Sweden

Received 17 January 1976

1. Introduction

There is now a great deal of evidence from a wide variety of secretory cells to support the idea that Ca^{2+} somehow links the process of stimulus recognition to the discharge of the secretory product. This has been considered to be true also for the pancreatic β-cells after the demonstration that omission of extracellular Ca^{2+} results in inhibition of the insulin-releasing action of glucose and other insulin secretagogues [1,2]. The fact that there is a need for extracellular Ca^{2+} might only indicate that a certain concentration of the ion is a prerequisite for a proper function of the secretory machinery. The idea of a direct regulatory role for Ca^{2+} should be amply supported if a rise of the extracellular concentration of this ion was found to stimulate the secretory activity in the absence of glucose or other initiators of insulin release. In the present communication evidence will be presented which indicate that this is the case and that the secretagogic action of excess extracellular Ca^{2+} can be inhibited by L-epinephrine and 2,4-dinitrophenol. Furthermore, it will be shown that the β-cells become more sensitive to extracellular Ca^{2+} when exposed to the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX).

2. Experimental

Female *ob/ob*-mice, 7 months old and taken from a non-inbred colony, were used as the source of pancreatic islets containing more than 90% β-cells [3]. The animals were starved overnight before being killed by decapitation. Fresh pancreatic islets were microdissected free-hand and subsequently incubated at 37°C with 1 mg/ml albumin and differert concentrations of Ca^{2+} in buffer equilibrated with oxygen. Preliminary incubation of batches of 2 islets for 40 min was followed by incubation for 60 or 90 min in 300 μl fresh medium. The basal incubation medium was a modified version of a Krebs-Ringer bicarbonate buffer with phosphate and sulphate replaced with equimolar amounts of chloride and the buffering capacity provided by 25 mM Hepes instead of bicarbonate. The concentration of Ca^{2+} was varied either by omitting the $CaCl_2$ or adding increasing concentrations of this salt up to 41.0 mM. When the resulting changes of the osmotic pressure were compensated for this was done either by reducing NaCl or adding choline Cl. After incubation the islets were freeze–dried and weighed as previously described [4]. The medium content of insulin was measured radioimmunologically using crystalline mouse insulin as a reference. Statistical differences were calculated from the mean differences between paired test and control incubations in a series of repeated experiments.

3. Results

Addition of $CaCl_2$ resulted in stimulation of insulin release irrespective of whether the changes of the osmotic pressure were compensated for or not by reducing NaCl (fig.1) or adding choline Cl (table 1). The effect of extracellular Ca^{2+} became more pronounced in the presence of the phosphodiesterase inhibitor IBMX. Ca^{2+} was now stimulatory at a lower concentration; maximal secretory response being reached at about 20 mM (fig.1). When raising the

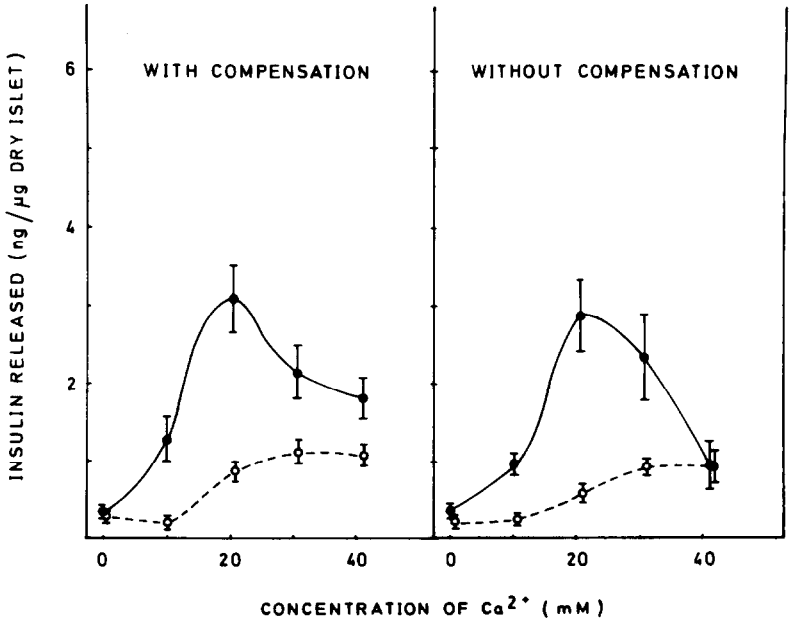

Fig.1. Calcium stimulation of insulin release in the presence (•) and absence (○) of 3-isobutyl-1-methylxanthine (IBMX). After 40 min of preliminary incubation with different concentrations of Ca^{2+} in the presence of 3 mM glucose and 1 mg/ml albumin the amounts of insulin released were recorded during 60 min of further incubation with or without 1 mM IBMX in the same type of basal medium lacking glucose. The increase of the osmotic pressure obtained by raising the Ca^{2+} concentration was either compensated for (left) or not (right) by reduction of NaCl. Mean values ± SEM for ng insulin released per µg islet dry weight in 8 separate experiments.

Table 1
Calcium stimulation of insulin release in the presence and absence of osmotic compensation by addition of choline Cl

Ca^{2+} conc. (mM)	IBMX conc. (mM)	Effect of raising Ca^{2+} from 2.6 mM	
		Without compensation	With compensation
20.5	0	+ 0.56 ± 0.11[c]	+ 0.51 ± 0.12[c]
41.0	0	+ 0.69 ± 0.22[b]	+ 0.58 ± 0.24[a]
20.5	1	+ 2.26 ± 0.42[c]	+ 1.89 ± 0.49[b]
41.0	1	+ 1.11 ± 0.38[b]	+ 0.74 ± 0.37

After 40 min of preliminary incubation with various concentrations of Ca^{2+} in the presence of 3 mM glucose and 1 mg/ml albumin the amounts of insulin released were recorded during 60 min of further incubation with or without 1 mM IBMX in the same type of basal medium lacking glucose. In the case the increase of the osmotic pressure resulting from the raise of Ca^{2+} was compensated for, this was done by adjusting the osmotic pressure by the addition of choline Cl to that found in the media with the highest concentration of Ca^{2+}. Mean values ± SEM for ng insulin released per µg dry weight in 8 separate experiments. [a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.005$.

Table 2
Inhibition of calcium-stimulated insulin release by L-epinephrine and 2,4-dinitrophenol

Ca^{2+} concn. (mM)	Test compound	Insulin release	Effect of test compound
2.6	–	0.30 ± 0.06	–
2.6	L-epinephrine	0.18 ± 0.07 (5)	– 0.10 ± 0.06 (5)
2.6	2,4-dinitrophenol	0.23 ± 0.06 (5)	– 0.09 ± 0.08 (5)
20.5	–	0.81 ± 0.19	–
20.5	L-epinephrine	0.19 ± 0.02	– 0.62 ± 0.20[b]
20.5	2,4-dinitrophenol	0.25 ± 0.07	– 0.56 ± 0.21[a]

After 40 min of preliminary incubation in media containing 3 mM glucose, 2.6 mM Ca^{2+} and 1 mg/ml albumin, the amounts of insulin released were recorded in the presence and absence of 2 μg/ml L-epinephrine or 0.3 mM 2,4-dinitrophenol during 90 min of further incubation with 2.6 or 20.5 mM Ca^{2+} in the same type of basal medium lacking glucose. Mean values ± SEM for ng insulin released per μg islet dry weight. When not otherwise stated within parenthesis 10 separate experiments were performed. [a] $P < 0.05$; [b] $P < 0.02$.

extracellular Ca^{2+} above this level the secretory rate progressively diminished. Both L-epinephrine and 2,4-dinitrophenol abolished the stimulation of insulin release obtained by excessive extracellular Ca^{2+} (table 2).

4. Discussion

It has recently been reported [5] that addition of 2–10 mM Ca^{2+} to a glucose-free bicarbonate medium results in short-lived release of insulin from rat pancreas when this has first been perfused with Ca^{2+}-deficient medium containing ethanedioxybis(ethylamine)tetra-acetic acid (EGTA). Since it was supposed that the pretreatment increased the Ca^{2+} permeability of the β-cell membrane the results were interpreted to indicate that Ca^{2+} might in itself trigger the release of insulin. The present study provides more direct evidence for a regulatory role of Ca^{2+} in showing that previous exposure to Ca^{2+}-deficient medium is no prerequisite for Ca^{2+} stimulation of insulin release. Essential for the success in these attempts was the use of a modified version of the Krebs-Ringer buffer where phosphate and sulphate were replaced with equimolar amounts of chloride and the buffering capacity provided by 25 mM Hepes instead of bicarbonate. With this modification it was possible to test concentrations of Ca^{2+} far above the limits of an ordinary bicarbonate medium.

In view of the great amounts of $CaCl_2$ added it was considered as important to check whether this initiated insulin release even when the changes of the osmotic pressure were compensated for either by reduction of NaCl or addition of choline chloride. This was found to be the case. It can consequently be assumed that the stimulation obtained is an effect of excess Ca^{2+} rather than the result of an increased osmotic pressure or excess Cl^-. In support for the idea that raising of the extracellular Ca^{2+} had initiated a physiological process the insulin secretory response was found to be inhibited by L-epinephrine and 2,4-dinitrophenol. Since these inhibitors are able to block a wide variety of stimuli they are supposed to operate at a late stage in the chain of events in the stimulus-secretion coupling [6]. It is of interest to note that Ca^{2+} is not unique among the alkaline-earth cations in initiating an insulin secretory response which can be blocked by L-epinephrine or 2,4-dinitrophenol. Milner and Hales [6] have clearly demonstrated that this is true also for Ba^{2+}.

It is well established that exposure to compounds which increase cAMP make the β-cells more sensitive to glucose and other physiological initiators of insulin release [7]. In support for that this holds also for stimulation with extracellular Ca^{2+}, this process was

found to be inhibited by L-epinephrine, a stimulator of the α-adrenergic receptor sites, and potentiated by IBMX, an inhibitor of the phosphodiesterase activity. Even if the present observations suggest that besides Ca^{2+}, cAMP is significant for the stimulussecretion coupling in the β-cells they provide no insight into the way by which the two factors are related. The secretory response to extracellular Ca^{2+} can for example be critically dependent on the β-cell level of cAMP both if the cyclic nucleotide mobilizes the ion from its non-regulatory storage sites or increases the sensitivity of the secretory machinery to the Ca^{2+} signal.

Previous studies of the β-cell-rich pancreatic mouse islets have indicated that extracellular Ca^{2+} is not only a prerequisite for the glucose stimulation of insulin release but that this process is inhibited by high concentrations of the divalent cation [8]. With the present observation that a further increase of the extracellular Ca^{2+} above 20 mM results in inhibition of insulin release when the glucose-free medium contains IBMX it became evident that the inhibitory component in the Ca^{2+} action involves the late stage of the secretory process. The fact that the Ca^{2+} action on insulin release seems to be the result of influences both from stimulatory and inhibitory pools of the ion complicates the attempts to a more detailed analysis of how Ca^{2+} regulates the β-cell function.

Acknowledgements

This study was supported by the Swedish Medical Research Council (12x-562). The author is indebted to the Swedish branch of Farbwerke Hoechst for providing the radioactive insulin used in the insulin assays.

References

[1] Grodsky, G. M. and Bennett, L. L. (1966) Diabetes 15, 910–913.
[2] Milner, R. D. G. and Hales, C. N. (1967) Diabetologia 3, 47–49.
[3] Hellman, B. (1965) Ann. N.Y. Acad. Sci. 131, 541–558.
[4] Hellman, B. (1970) Diabetologia 6, 110–120.
[5] Devis, G., Somers, G. and Malaisse, W. J. (1975) Biochem. Biophys. Res. Commun. 67, 525–529.
[6] Milner, R. D. G. and Hales, C. N. (1969) Biochem. J. 113, 473–479.
[7] Grodsky, G. M. (1970) Vitamins and Hormones 28, 37–101.
[8] Hellman, B. (1975) Endocrinology 97, 392–398.