Insulin release has no absolute requirement for extracellular monovalent ions

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Current chemiosmotic models of exocytosis ascribe an essential role to the influx of extracellular monovalent anions or cations into the secretory granules apposed to the plasma membrane. These hypotheses were tested by measuring insulin release in sucrose media devoid of monovalent ions. A small response to glucose (25% of controls) was still observed, which could be potentiated by isobutylmethylxanthine and suppressed by cobalt or low temperature. Substitution of Ba^{2+} for Ca^{2+} triggered a practically normal release of insulin that was inhibited by blockers of Ca^{2+} channels (cobalt or D 600) and abolished by low temperature. These results show that insulin release remains possible in the absence of extracellular monovalent ions and, therefore, that the chemiosmotic models of exocytosis do not entirely apply to insulin release.

(Pancreatic islet) Insulin release Ion influx Exocytosis Chemiosmotic model

1. INTRODUCTION

Considerable evidence has established the importance of ions for the control of insulin release by pancreatic B-cells (reviews [1–3]). Though changes in ionic fluxes are mainly involved in coupling recognition of the secretagogue to activation of the effector system leading to exocytosis, they may also participate in other steps of the secretory sequence.

A chemiosmotic model has been proposed [4] to account for the exocytotic release of various secretory products. According to this hypothesis, anion transport sites are formed during fusion of secretory granule and plasma membranes. They permit influx of anions (mainly Cl^-) into the granule, down an electrochemical gradient created by the H^+ pump present in the granule membrane. The resulting increase in osmotic pressure within the granule leads to swelling and rupture at the site of membrane contact. An alternative to this original model ascribes the osmotic role to cations (mainly Na^+), which would exchange with intragranular H^+. Replacement of outgoing H^+ by H^+ released from intragranular buffers fosters the exchange process and also leads to an osmotic swelling [5].

The inhibition of insulin release in hyperosmolar or in low-Cl^- solutions [6–9] may suggest that exocytosis of insulin granules conforms to the first of these models. However, both experimental conditions also alter earlier steps of the stimulus-secretion coupling [9,10], and the requirement for extracellular Cl^- varies with the stimulus [9]. On the other hand, the importance of extracellular Na^+ for optimal insulin release by intact [11] or permeabilized [12] islets could be interpreted as support for the second of the above models.

To obtain more conclusive evidence for or against the participation of chemiosmotic mechanisms in the ultimate step of insulin release, we have examined whether this latter remains possible in a sucrose medium, devoid of monovalent ions.

2. MATERIALS AND METHODS

All experiments were performed with islets

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isolated by collagenase digestion of the pancreas of fed female NMRI mice. Immediately after isolation the islets were placed in the chambers of a dynamic system of perifusion [13]. The immunoreactive insulin content of the effluent fractions was measured [14] with rat insulin as standard.

The control salt-balanced medium had the following composition (in mM): 133 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 10.5 Hepes and 5 NaOH. In certain experiments $CaCl_2$ was replaced by $BaCl_2$. The sucrose medium contained (in mM): 290 sucrose, 2.5 $Ca(OH)_2$ and 10.5 Hepes. In certain experiments $Ca(OH)_2$ was replaced by $Ba(OH)_2$. All solutions had a pH of 7.4 at 37°C, were continuously gassed with O_2 and supplemented with bovine serum albumin (1 mg/ml). Unless otherwise specified, the concentration of glucose was 5 mM. Isobutylmethylxanthine was obtained from Aldrich Europe (Brussels) and the Ca^{2+}-channel blocker α-isopropyl-α-[(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl]-3,4,5-trimethoxyacetonitrile hydrochloride (D 600) from Knoll (Ludwigshafen, FRG). All other reagents were from Merck (Darmstadt).

Results are presented as means (± SE) for a certain number of experiments performed with different islet preparations.

3. RESULTS

Replacement of the salt-balanced medium by a sucrose medium only caused a slight and transient increase in insulin release when the concentration of glucose remained at 5 mM (fig.1). Raising the concentration of glucose to 20 mM in the sucrose medium stimulated insulin release. However, compared to the control response observed in the salt-balanced medium, this stimulation was slow, small (25%) and not well sustained (fig.1). This effect of glucose was abolished by 1 mM cobalt (not shown).

Glucose-induced insulin release was potentiated by isobutylmethylxanthine (fig.2). This potentiation was relatively larger in the sucrose medium (2.4-fold) than in the salt-balanced medium (1.6-fold). The response to the combination of glucose and isobutylmethylxanthine was reversible and was totally suppressed if the experiments were carried out at 20°C. The small increase in release

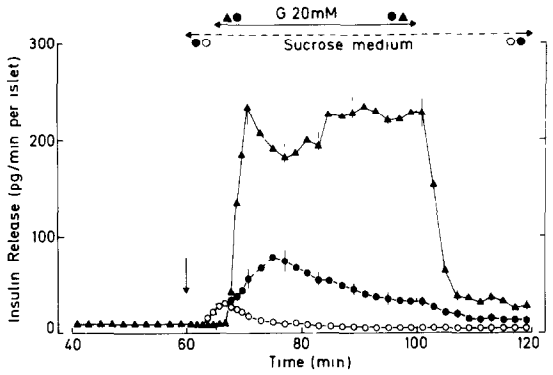

Fig.1. Insulin release by perifused mouse islets stimulated by glucose in a sucrose medium. All experiments started in a control, salt-balanced medium containing 5 mM glucose. In one series (▲) the ionic composition of the medium was not changed, but the concentration of glucose (G) was raised to 20 mM between 65 and 100 min. In the other series, the control medium was replaced by a sucrose medium from 60 min onwards. The concentration of glucose was either maintained at 5 mM until the end (○) or raised to 20 mM between 65 and 100 min (●). Values are means ± SE for 5 experiments.

occurring upon substitution of the sucrose medium for the control medium was also abolished at low temperature (fig.2).

When Ca^{2+} was replaced by Ba^{2+} in a salt-balanced medium, the rate of insulin release markedly increased to a peak reached after 7 min, and then slowly declined with time (fig.3). The same substitution in a sucrose medium also triggered insulin release. Though the initial peak was 35% lower, the late phase was similar to that observed in control islets. This stimulation by Ba^{2+} was reversible upon return to a Ca^{2+} medium and was abolished by performing the experiments at 20°C (fig.3).

Cobalt totally prevented Ba^{2+} from stimulating insulin release in a sucrose medium (fig.4). This inhibitory effect was completely reversible upon withdrawal of cobalt. On the other hand, D 600 only caused a partial and delayed inhibition of the insulin response to Ba^{2+}, and this inhibitory effect was not reversible upon withdrawal of D 600. Cobalt, but not D 600, also suppressed the transient release of insulin occurring when the salt-balanced medium is replaced by a sucrose medium (fig.4).

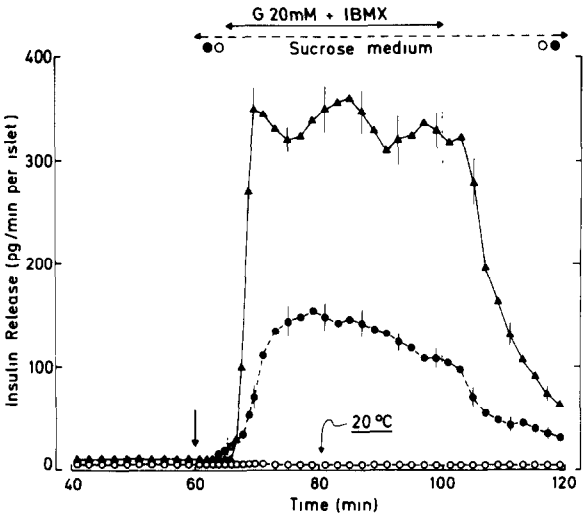

Fig.2. Insulin release by perifused mouse islets stimulated by glucose and isobutylmethylxanthine (IBMX) in a sucrose medium. All experiments started in a control, salt-balanced medium containing 5 mM glucose. In all series the concentration of glucose was raised to 20 mM and IBMX (1 mM) was added to the medium between 65 and 100 min. In two series (●,○) the control medium was replaced by a sucrose medium from 60 min onwards. One series of experiments was performed at 20°C throughout (○). Values are means ± SE for 4–6 experiments.

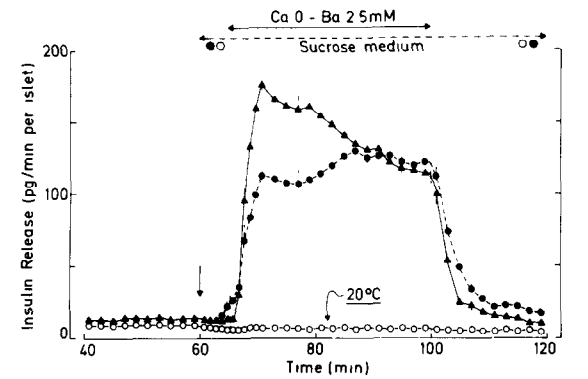

Fig.3. Insulin release by perifused mouse islets stimulated by barium in a sucrose medium. The concentration of glucose was 5 mM throughout. All experiments started in a control, salt-balanced medium. In all series, barium was substituted for calcium between 65 and 100 min. In two series (●,○) the control medium was replaced by a sucrose medium from 60 min onwards. One series of experiments was performed at 20°C throughout (○). Values are means ± SE for 4–6 experiments.

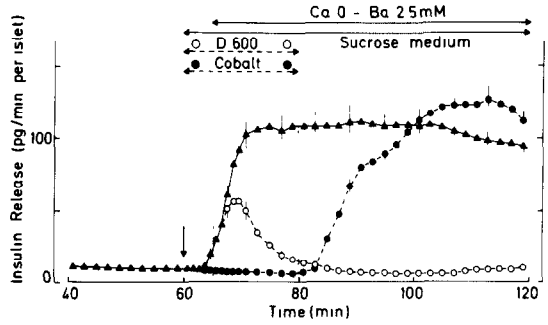

Fig.4. Inhibition by D 600 and cobalt of insulin release stimulated by barium in mouse islets perifused with a sucrose medium. The concentration of glucose was 5 mM throughout. In all series, the control, salt-balanced medium was replaced by a sucrose medium from 60 min onwards, and barium was substituted for calcium from 65 min onwards. In two series, either 50 μM D 600 (○), or 1 mM cobalt (●) was added to the medium between 60 and 80 min. Values are means ± SE for 5 experiments.

4. DISCUSSION

A number of experiments have shown that changes in the extracellular concentration of individual monovalent ions (Na^+, K^+, Cl^-, HCO_3^-) alter the B-cell response to physiological secretagogues (review [3]). The inhibition of the response to glucose observed here in a sucrose medium further supports the importance of these ions for a normal functioning of B-cells. However, the essential information provided by this study is that some stimulation of insulin release remains possible in the nominal absence of extracellular monovalent cations and anions. The cellular loss of ions certainly cannot lead to significant accumulation in the intercellular space for long periods. Thus, extracellular space markers are completely washed out from the islets in less than 10 min in this dynamic system of perifusion [15]. Moreover, Hepes cannot substitute for physiological ions since it does not pass through biological membranes [16].

No substantial leak of insulin from B-cells was caused by substitution of a sucrose medium for the control salt-balanced medium. The small and transient initial rise in secretion rate was suppressed by cobalt or by low temperature. Glucose was still able to trigger insulin release in the sucrose

medium. Its effect could be amplified by isobutylmethylxanthine, which potentiates release by raising cAMP levels and by directly mobilizing cellular calcium (review [17]). On the other hand, it was abolished by lowering the temperature. In control solutions, the inhibitory effect of cooling is currently ascribed to inhibition of glucose metabolism in B-cells and to interference with a still unidentified step between the rise in cytoplasmic Ca^{2+} and the exocytosis of insulin granules [18].

Ba^{2+}-induced insulin release is due to the ability of this divalent cation to enter B-cells through verapamil-inhibitable channels [19] and to mobilize cellular calcium [20]. The insulinotropic effect of Ba^{2+} was only slightly and transiently depressed in a sucrose medium. Its complete reversibility, its abolition by low temperature and its inhibition by the Ca^{2+}-channel blockers cobalt and D 600 argue against an abnormal, uncontrolled release of insulin under these conditions.

In spite of expected [3] quantitative differences, the release of insulin induced by glucose or by Ba^{2+} in a sucrose medium shows enough similarities with control insulin release to permit the conclusion that it occurs by a similar basic mechanism. It appears, therefore, that monovalent ions are not a prerequisite for insulin exocytosis, and that neither of the proposed chemiosmotic models entirely applies to insulin release. Observations that catecholamine release by intact [21] or permeabilized chromaffin cells [22,23] is not abolished in sucrose media have also led to a similar conclusion for exocytosis of chromaffin granules. It is not excluded that osmotic events participate in exocytosis [10,21,22], but further experiments are required to determine their possible nature and mechanisms.

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