STIMULATION OF INSULIN RELEASE BY CALCIUM[1]

Ghislain Devis, Guido Somers and Willy J. Malaisse

Laboratory of Experimental Medicine, Brussels University School of Medicine, Brussels, Belgium

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SUMMARY: In the absence of secretagogue, Ca^{2+} (2 to 10 mM) provokes a short-lived release of insulin in the perfused rat pancreas first exposed to EGTA. The secretory response is abolished by verapamil and enhanced by theophylline. These findings afford the first demonstration that Ca^{2+} itself can trigger insulin release.

The release of insulin evoked by glucose and other secretagogues in the endocrine pancreas is thought to be mediated through an accumulation of Ca^{2+} in some critical site of the B-cell (1). This concept is based mainly on the dependency of the secretory response to extracellular Ca^{2+} (2, 3), and on the influence of insulinotropic agents upon Ca^{2+} handling by isolated islets of Langerhans (4-7). We now wish to demonstrate that Ca^{2+} itself may indeed promote insulin release.

METHODS

Pancreases were removed from fully fed albino rats and placed in an open circuit extracorporeal perfusion unit. The perfusate was a bicarbonate-buffered solution containing albumin (40 mg/ml), heparinized rat blood (10 %, v/v) and, as required, EGTA (ethyleneglycol-bis-(β-amino-ethyl ether)N,N'-tetra-acetic acid neutralized to pH 7 with NaOH), verapamil (Knoll A.G.) and theophylline. The surgical and experimental procedures and immunoassay method for insulin are detailed elsewhere (8). The rate of insulin secretion is expressed as μU/min per pancreas.

RESULTS AND DISCUSSION

After pretreatment for 24 min with a medium deprived of Ca^{2+} and enriched

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with EGTA (4 mM), the pancreases were exposed to EGTA-free perfusates containing Ca^{2+} in concentrations between 2 and 10 mM. A dramatic but short-lived secretory response was observed in response to Ca^{2+} administration (Figure 1, left panel). The magnitude of the secretory response correlated with the Ca^{2+} concentration of the perfusate. The release of insulin evoked by calcium (10 mM) was suppressed in the presence of verapamil (Figure 1, middle panel), which inhibits Ca^{2+} inward transport in the B-cell (9-11). Prior exposure of the pancreas to a sufficient concentration of the Ca^{2+}-chelating agent appeared as a critical feature of our experimental design. Indeed, the secretory response to Ca^{2+} (10 mM) was abolished if, during the pretreatment period, EGTA was absent, used in low concentration (1 mM), or present in the perfusate in high concentration (4 mM) together with a large amount of Ca^{2+} (Figure 1, right

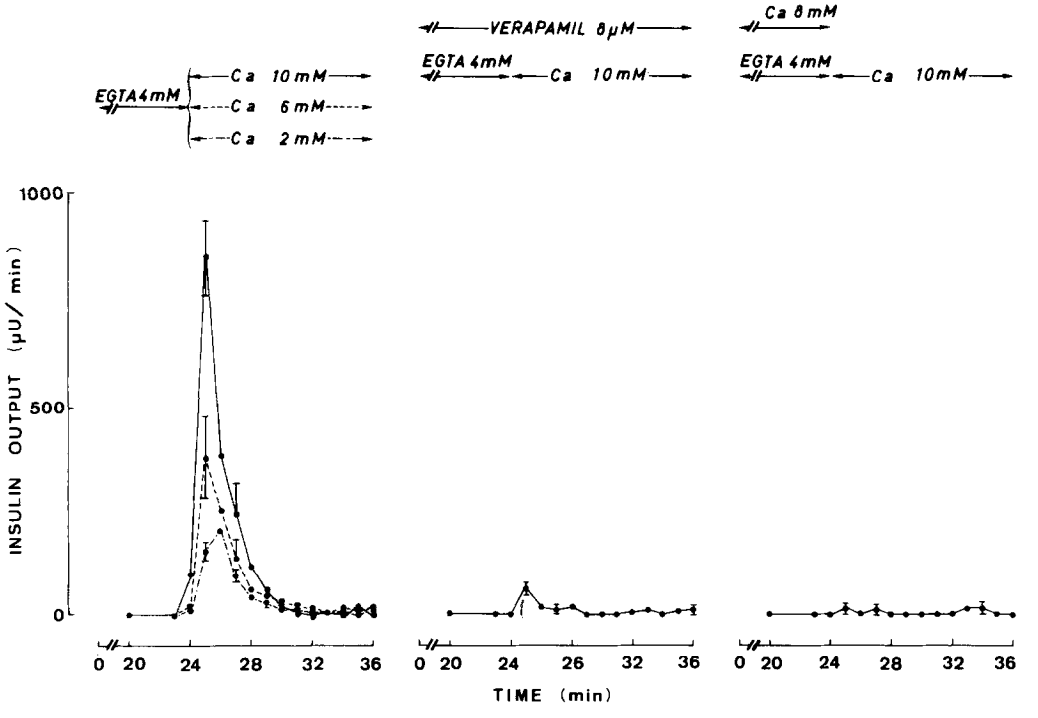

Figure 1: Effect of Ca^{2+} upon insulin release by the isolated perfused rat pancreas pretreated with EGTA (4 mM). Mean values (± s.e.m.) refer to 2 to 4 individual experiments in each group.

panel ; Figure 2, left panel). Theophylline (1.4 mM), which is thought to provoke a glucose-independent intracellular translocation of Ca^{2+} in the B-cell (6, 12), enhanced the secretion of insulin evoked by Ca^{2+} (10 mM) after pretreatment with EGTA. In the presence of theophylline, pretreatment of the pancreas with as little as 0.5 mM EGTA was sufficient to permit stimulation of insulin release in response to Ca^{2+} administration.

The procedure used in the present study to demonstrate the insulinotropic action of Ca^{2+} is analogous to that used by Douglas and Rubin to stimulate catecholamine release in chromaffin cells (13). Ca^{2+} depletion, as provoked by

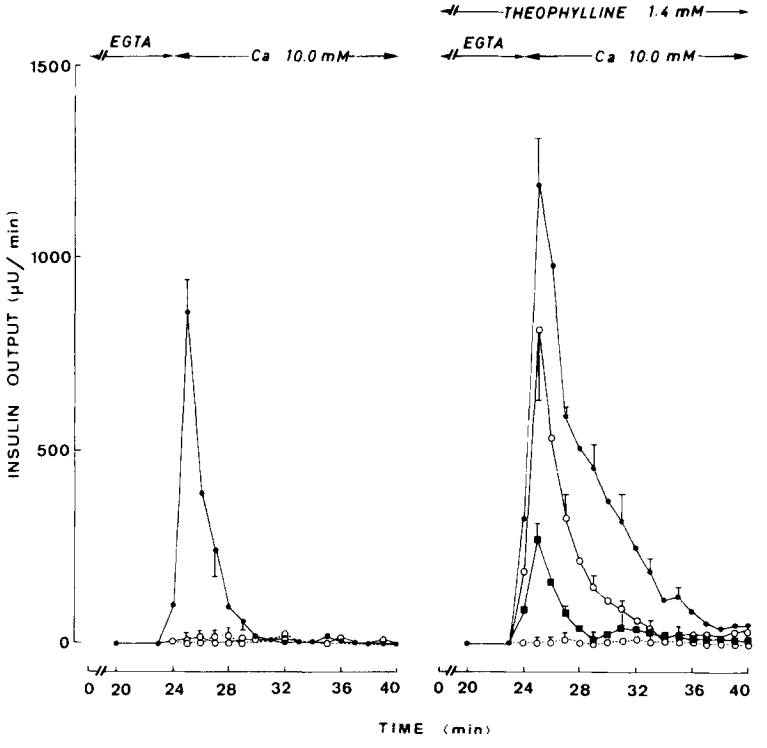

Figure 2: Effect of Ca^{2+} in the absence (left panel) or presence (right panel) of theophylline (1.4 mM) upon insulin release by the isolated perfused rat pancreas. During the first 24 min, the Ca^{2+}-deprived media either contained no EGTA (open circles and dotted line) or was enriched with EGTA in various concentrations : 0.5 mM (closed squares and solid line), 1.0 mM (open circles and solid line) and 4.0 mM (closed circles and solid line). Mean values (± s.e.m.) refer to 2 to 5 individual experiments in each group.

EGTA, may permeate the cell membrane and facilitate Ca^{2+} influx. In turn, the briefness of the secretory response could be due to rapid restoration of normal membrane permeability. The inhibitory effect of verapamil suggests that, even after treatment with EGTA, the influx of Ca^{2+} occurs through the same channels as those operative at normal Ca^{2+} concentration.

So far, only indirect evidence was available to support the idea that Ca^{2+} can trigger insulin release in the absence of an insulinotropic agent. Short-lived release of insulin is provoked by high concentrations of K^+ known to cause cell depolarization, the secretory response being abolished in the absence of extracellular Ca^{2+} (2, 14). Ba^{2+} also causes a transient release of insulin, provided that Ca^{2+} is omitted from the medium (5, 15, 16). Sustained stimulation of insulin release in the absence of glucose and presence of Ca^{2+} is seen either in pieces of pancreas incubated in alkalotic media deprived of Mg^{2+} and enriched with K^+ (17), or in monolayer culture of neonatal pancreas exposed to ionophore A23187 (18). The present results indicate, for the first time, that Ca^{2+} itself can trigger the release of insulin.

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