CALCIUM ANTAGONISTS AND ISLET FUNCTION.
IX. IS EXTRACELLULAR CALCIUM REQUIRED FOR INSULIN RELEASE?

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Although the dependency of insulin release on extracellular Ca\(^{2+}\) is well documented \(^8,13\), conflicting views were recently expressed concerning the precise role of extracellular Ca\(^{2+}\) in the biphasic insulin secretory response evoked in the endocrine pancreas by a square-waved increase in glucose concentration \(^7,20\). It is agreed, however, that removal of extracellular Ca\(^{2+}\) abolishes the sustained secondary phase of glucose-induced insulin release, which may well represent the physiologically most relevant component of the secretory response \(^4\). Removal of extracellular Ca\(^{2+}\) also abolishes the glucose-induced electrical activity \(^1\), which is thought to be directly related to the process of insulin release \(^14\). However, the electrical activity of the B-cell is apparently not suppressed in the absence of Ca\(^{2+}\) when Mg\(^{2+}\) is also removed from the extracellular milieu \(^1\). In the preceding report in this series, it was shown that the well-known inhibitory effect of high concentrations of Mg\(^{2+}\) (5-10 mM) upon glucose-induced insulin release \(^2,5,10,13\) is mainly due to a decrease in the rate of Ca\(^{2+}\) entry into the islet cells \(^11\). The present work, part of which was reported in abstract form \(^16\), aims at a re-evaluation of the significance of extracellular Ca\(^{2+}\) and Mg\(^{2+}\), at physiological concentrations, in the process of glucose-stimulated insulin secretion.

MATERIALS AND METHODS

The dynamic aspects of glucose-induced insulin release, at different concentrations of Ca\(^{2+}\) and Mg\(^{2+}\), were investigated in the isolated perfused rat pancreas preparation, which is described in detail elsewhere \(^19\). The perfusate contained dextran (40 mg/ml) and bovine albumin (5 mg/ml) as previously reported \(^8\). When no

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Ca\textsuperscript{2+} or Mg\textsuperscript{2+} was added to the perfusate, the total concentration of these elements, as measured by atomic absorption, averaged 0.35 ± 0.05 mM (n = 31) and 0.05 ± 0.01 mM (n = 6) for Ca and Mg, respectively. The concentration of ionized Ca\textsuperscript{2+} and Mg\textsuperscript{2+} was not measured. The above-mentioned values for the total concentration of Ca and Mg are in fair agreement with the measurements performed when Ca\textsuperscript{2+} and Mg\textsuperscript{2+} (1.0 mM each) were added to the perfusate, in which case the total measured concentration of Ca and Mg averaged 1.27 ± 0.02 mM (n = 25) and 0.90 ± 0.02 mM (n = 4), respectively. In the results and discussion sections, the quoted concentrations of Mg\textsuperscript{2+} and Ca\textsuperscript{2+} invariably refer to the amount of Mg\textsuperscript{2+} and Ca\textsuperscript{2+} added to the perfusate.

The steady-state (or integrated) values for insulin release were measured by incubating groups of 8 isolated islets each for 90 min in 1.0 ml of medium containing bovine albumin (5 mg/ml), as described in full detail elsewhere\textsuperscript{10}.

The results are always expressed as the mean (± SEM), together with the number of individual determinations (n).

RESULTS AND COMMENTS

1. Effect of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} upon the initial secretory response to glucose

In the presence of 2.0 mM Ca\textsuperscript{2+} and 1.0 mM Mg\textsuperscript{2+}, the introduction of 16.7 mM glucose at the 25th min of perfusion provoked the usual pattern of insulin release (fig. 1). The initial peak output was reached at the 27th min, and a slowly rising second phase of release was observed from the 31st min onwards. When no Ca\textsuperscript{2+} was added to the perfusate throughout the experiment, a modest and delayed initial response to glucose was noticed, but the rate of secretion during the late phase

![Fig. 1 - Effect of glucose upon the output of insulin from the isolated perfused rat pancreas. The perfusate administered throughout the experiment contained 2.0 mM Ca\textsuperscript{2+} and 1.0 mM Mg\textsuperscript{2+} (dashed line; n = 10), no Ca\textsuperscript{2+} and 1.0 mM Mg\textsuperscript{2+} (open circles; n = 3), or neither Ca\textsuperscript{2+} nor Mg\textsuperscript{2+} (closed triangles; n = 3).](image-url)