MATHEMATICAL MODELING OF STIMULUS-SECRETION COUPLING IN THE PANCREATIC β-CELL

III. Glucose-induced Inhibition of Calcium Efflux

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ABSTRACT The inhibitory effect of glucose upon ^{45}Ca efflux from prelabeled pancreatic islets was simulated in a mathematical model for Ca^{2+}-cyclic AMP interaction in the process of glucose-induced insulin release. At variance with a previous interpretation, it was postulated that glucose inhibits ^{45}Ca efflux by facilitating the uptake of the cation by the vacuolar system. The latter facilitation did not hinder glucose from provoking a rapid accumulation of cytosolic Ca^{2+} and, hence, insulin release. The postulated facilitation was also suitable in simulating the effect of glucose upon ^{45}Ca efflux, uptake, and intracellular distribution in the pancreatic islets.

INTRODUCTION

In the present series of reports, a mathematical model for the interaction between Ca and cyclic AMP in the control of insulin secretion (1) was modified to simulate the biphasic pattern of glucose-induced insulin release (2) and to mimic the Ca-dependent stimulation by glucose of ^{45}Ca efflux from prelabeled islets (Scholler, Y., V. De Maertelaer, and W. J. Malaisse, manuscript submitted for publication). For such purposes, it was postulated that (*a*) the cytosolic and vacuolar Ca pools in the islet cells are stratified in a cortical layer and central core, (*b*) the stimulant action of glucose on Ca inflow into the islet cells is a discontinuous phenomenon, and (*c*) the fractional rate of Ca release by the vacuolar system is regulated by the cytosolic Ca concentration in a process of Ca-stimulated Ca release (2, Scholler, Y., V. De Maertelaer, and W. J. Malaisse, manuscript submitted for publication). The aim of the present work is to reevaluate the significance of the second major effect of glucose upon ^{45}Ca handling, namely, the inhibition of ^{45}Ca efflux from prelabeled islets. Such an inhibitory effect represents an early and sustained phenomenon that, however, is masked by a secondary rise in ^{45}Ca fractional outflow rate when the islets are perifused in the presence of extracellular Ca^{2+} (3, 4). Two distinct explanations have been proposed to account for such an inhibitory effect of glucose on ^{45}Ca outflow. First, the glucose-induced decrease in ^{45}Ca outflow could reflect a true decrease in the efficiency of Ca outwards transport across the plasma membrane, e.g., by inhibition of a process of Na-Ca countertransport (5, 6). Second, the decrease in ^{45}Ca outflow could be secondary to a glucose-induced stimulation of Ca uptake by intracellular organelles belonging to the vacuolar system (7). Such a stimulation, which could be linked to an increase in ATP generation rate, would result in an initial decrease in the cytosolic Ca^{2+} concentration and, according to some authors (8, 9), may be responsible for the inactivation by glucose of a Ca^{2+}-sensitive modality of K^+ extrusion from the islet cells. This decrease in K^+ conductance could then lead to cell membrane depolarization and the gating of voltage-sensitive Ca^{2+} channels (8, 10). Our mathematical modeling of stimulus-secretion coupling in the pancreatic β-cell was previously based on the assumption that glucose inhibits the fractional transport rate of Ca from the cytosol across the plasma membrane into the extracellular fluid. In the present study, however, the opposite view is taken; glucose is assumed to increase the fractional removal rate of cytosolic Ca by the vacuolar system.

MATERIALS AND METHODS

The initial model used in these experiments was previously defined (see Model V in reference 1). In the present series of experiments, however, it was postulated, at variance with our initial model, that glucose fails to affect the fractional outflow rate of Ca from the cytosol into the extracellular fluid. Such a fractional outflow rate was maintained constant at 0.0462 min^{-1}, which is the value chosen in our initial model for glucose-deprived islets (1). When the cytosolic and vacuolar Ca pools were stratified in a cortical layer (representing 5% of the total cellular pool) and a central core, the fractional outflow rate of Ca from the cortical layer into the extracellular fluid was increased to 0.9240 min^{-1} in inverse proportion to the relative size of such a cortical layer, so that under steady state conditions the rate of Ca^{2+} outflow from glucose-deprived islets would remain equal to the rate of Ca^{2+} inflow into the islets (2).

In all the present experiments, glucose was assumed to enhance the fractional removal rate of cytosolic Ca by the vacuolar system from a basal value of 0.0097 to 0.0400 min^{-1}. The latter value was selected because it resulted in a glucose-induced decrease in ^{45}Ca outflow of the

TABLE I

EFFECT OF GLUCOSE UPON THE STEADY STATE VALUES FOR CYTOSOLIC Ca CONTENT (Ca_{cy})* AND CYCLIC AMP CONTENT (Y)‡ OF PANCREATIC ISLETS, AND THE CORRESPONDING FRACTIONAL OUTFLOW RATES OF Ca FROM THE VACUOLAR SYSTEM (P)§

Glucose	No glucose	16.7 mM
Ca_{cy} (pmol/islet)	2.5974	5.9524
Y (fmol/islet)	7.8	15.6
Eq. 1 $P = 0.0025025\ Ca_{cy}$	0.0065	0.0149
Eq. 2 $P = 0.0009634\ (Ca_{cy})^2$	0.0065	0.0341
Eq. 3 $P = 0.0028358\ e^{0.1063Y}$	0.0065	0.0149
Eq. 4 $P = 0.0012375\ e^{0.2126Y}$	0.0065	0.0341

Also shown are the four equations (Eqs. 1–4) used here in this study to define P as a function of Ca_{cy} or Y.

*See Table III.

‡See reference 1.

§P is expressed as min^{-1}.

same magnitude as that seen in an earlier study, when glucose was assumed to decrease the fractional outflow rate of Ca from the cytosol into the extracellular fluid from a basal value of 0.0462 to 0.0293 min^{-1}.

Four different modalities were considered to rule the fractional outflow rate of Ca from the vacuolar pool. In the first and second modalities, such a fractional outflow rate (P, expressed as min^{-1}) was related to the Ca concentration of the corresponding cytosolic area (Ca_{cy}, expressed as picomole per islet) by one of the two following equations:

$$P = 0.0025025\ Ca_{cy} \quad (1)$$

$$P = 0.0009634\ (Ca_{cy})^2. \quad (2)$$

The selection of these two equations and their adaptation to the stratified model were considered in detail in an earlier work (Scholler, Y., V. De Maertelaer, and W. J. Malaisse, manuscript submitted for publication). In the third and fourth modalities, P was regulated by the islet content in cyclic AMP (Y, expressed as femtomole per islet), according to one of the two following equations

$$P = 0.0028358\ e^{0.1063Y} \quad (3)$$

$$P = 0.0012375\ e^{0.2126Y}. \quad (4)$$

These equations imply that cyclic AMP stimulates the release of Ca from the vacuolar system (11, 12). As illustrated in Table I, Eqs. 3 and 4 were established so that they would yield the same value for P as those derived

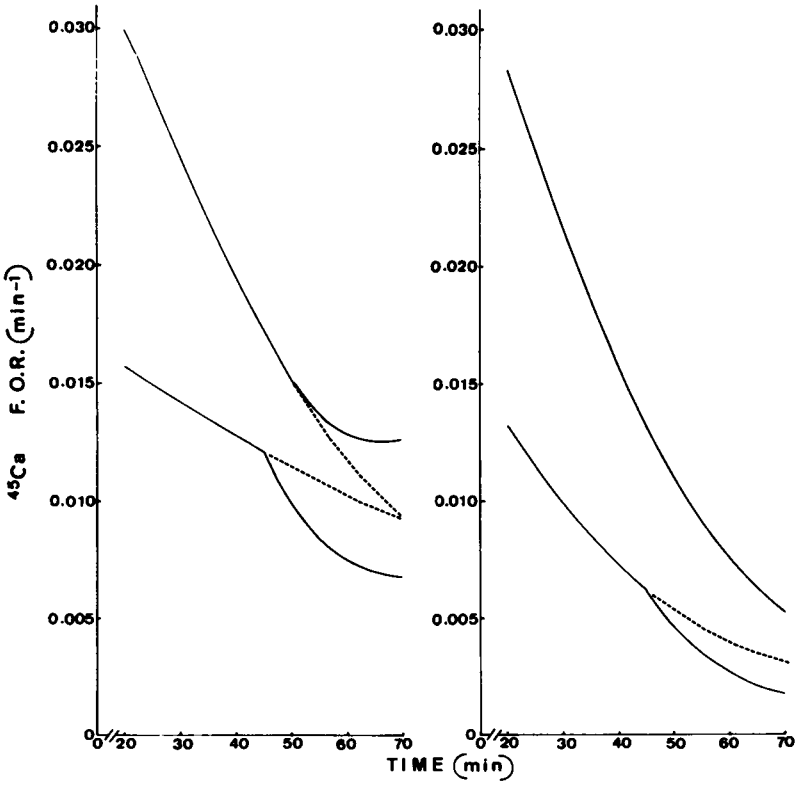

FIGURE 1 Time course for ^{45}Ca fractional outflow rate from islets preincubated for 60 min in the presence of glucose (16.7 mM) and $^{45}Ca^{2+}$ (1.0 mM) and perifused from time 0 to 45 min in the absence of glucose and from the 45 min onwards in the presence of glucose (16.7 mM). The perifusive either contained $^{40}Ca^{2+}$ (1.0 mM; *left*) or was deprived of Ca^{2+} (*right*). The results refer to an unstratified model in which glucose, in addition to increasing the inflow rate of Ca^{2+} from 0.120 to 0.275 pmol/islet per min, either failed to affect the fractional removal rate of cytosolic Ca by the vacuolar system (*upper* curves) or increased such a fractional removal rate from 0.0097 to 0.04 min^{-1} (*lower* curves). In all cases, the fractional rate of Ca release by the vacuolar system was ruled by the cyclic AMP content of the islets according to Eq. 4. The dashed lines (---) refer to control experiments performed throughout the 70 min of perifusion in the absence of glucose. F.O.R. stands for the fractional outflow rate.

from Eqs. 1 and 2, respectively, whether in glucose-deprived or glucose-stimulated islets examined under steady state conditions.

In each individual simulation, the equations ruling the movements of Ca in the islet cells were identical during the preincubation (60 min) of the islets in the presence of glucose (16.7 mM) and ^{45}Ca (1.0 mM) and during perfusion (70 min) of the islets by nonradioactive media.

The rate of insulin release was regulated by both the outer cytosolic Ca concentration and islet content in cyclic AMP according to an equation previously defined (see Eq. 8 in reference 1). All phenomena described in the Results section refer to theoretical data derived from the mathematical model.

RESULTS

Fig. 1 indicates that an increase in the fractional removal rate of cytosolic Ca by the vacuolar system in response to glucose administration caused an immediate and sustained decrease in ^{45}Ca fractional outflow rate (expressed as the fraction of ^{45}Ca released per minute by prelabeled islets relative to their actual ^{45}Ca content) in the unstratified model. In Fig. 1, the pattern of ^{45}Ca efflux in response to glucose stimulation was not vastly different in the absence or presence of extracellular Ca^{2+} provided that glucose indeed increased the fractional removal rate of cytosolic Ca by the vacuolar system. In this model, the fractional rate of Ca release from the vacuolar system was ruled by the cyclic AMP content of the islets. In the unstratified model, however, essentially the same results were obtained when the fractional rate of Ca release by the vacuolar system was assumed proportional to either the cytosolic Ca concentration or the square of such a concentration (Fig. 2). Indeed, under such conditions, and even in the presence of extracellular Ca^{2+}, the overall effect of glucose remained to decrease ^{45}Ca outflow (Fig. 2).

A different picture was obtained in the stratified model (Fig. 3). In this case, glucose caused an obvious stimulation of ^{45}Ca outflow provided that (*a*) Ca^{2+} was present in the perifusion medium and (*b*) the rate of Ca exchange

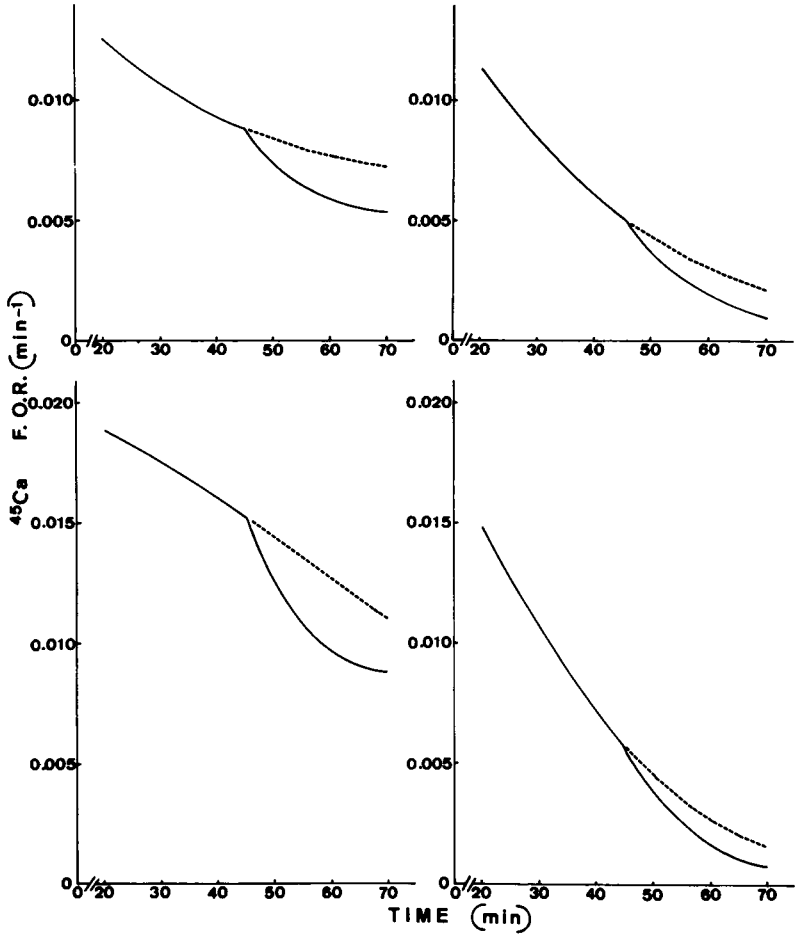

FIGURE 2 Time course for ^{45}Ca fractional outflow rate from islets preincubated for 60 min in the presence of glucose (16.7 mM) and $^{45}Ca^{2+}$ (1.0 mM) and perifused from time 0 to 45 min in the absence of glucose and from the 45 min onwards in the presence of glucose (16.7 mM). The perifusive either contained $^{40}Ca^{2+}$ (1.0 mM; *left*) or was deprived of Ca^{2+} (*right*). The results refer to an unstratified model in which glucose, in addition to increasing the inflow rate of Ca^{2+} from 0.120 to 0.275 pmol/islet per min, also increased the fractional removal rate of cytosolic Ca by the vacuolar pool from 0.0097 to 0.04 min^{-1}. The fractional rate of Ca release by the vacuolar system was proportional to either the cytosolic Ca concentration (*upper* curves) or the square of such a concentration (*lower* curves). The dashed lines (---) refer to control experiments performed throughout the 70 min of perifusion in the absence of glucose. F.O.R. stands for the fractional outflow rate.

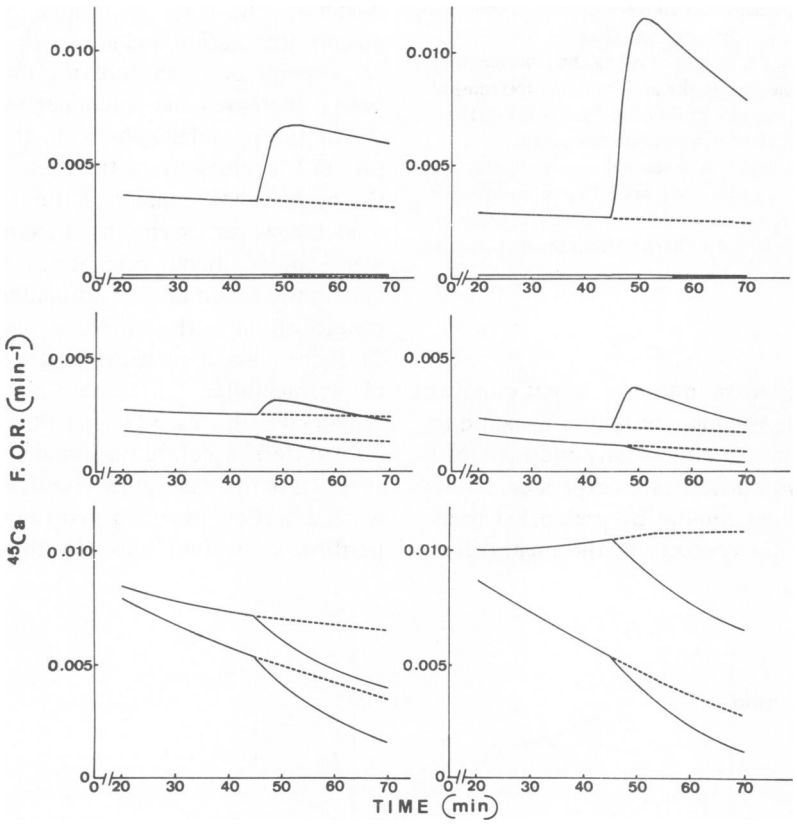

FIGURE 3 Time course for ^{45}Ca fractional outflow rate from islets preincubated for 60 min in the presence of glucose (16.7 mM) and ^{45}Ca (1.0 mM) and perifused from time 0 to 45 min in the absence of glucose and from the 45 min onwards in the presence of glucose (16.7 mM). The perifusive either contained $^{40}Ca^{2+}$ (1.0 mM; *upper* curve in each panel) or was deprived of Ca^{2+} (*lower* curve in each panel). The results refer to a stratified model in which the peripheral cytosolic (or vacuolar) pool represented 5% of the total cytosolic (or vacuolar) pool. The fractional outflow rate of cytosolic Ca from the inner to outer pool amounted to 0.001 (*upper*), 0.007 (*middle*), and 0.06 min^{-1} (*lower*), respectively. In all cases, glucose, in addition to increasing the inflow rate of Ca^{2+} from 0.120 to 0.275 pmol/islet per min, augmented the fractional removal rate of cytosolic Ca by the vacuolar system from 0.0097 to 0.04 min^{-1}. The fractional rate of Ca release by the vacuolar system was proportional to either the cytosolic Ca concentration (*left*) or the square of such a concentration (*right*). The dashed lines (---) refer to control experiments performed throughout the 70 min of perifusion in the absence of glucose. F.O.R. stands for the fractional outflow rate.

between the peripheral and central cytosolic pools remained sufficiently low. For instance, when the fractional outflow rate of cytosolic Ca from the inner to outer pool amounted to 0.007 min^{-1}, glucose augmented ^{45}Ca outflow in the presence of extracellular Ca^{2+} and instead decreased ^{45}Ca outflow in the absence of extracellular Ca^{2+} (Fig. 3, middle panels). Thus, in the stratified model, it was possible to simulate the dual effect of glucose upon ^{45}Ca outflow, even when glucose stimulated the uptake of Ca by the vacuolar system.

A comparable dissociation between a stimulatory effect of glucose upon ^{45}Ca outflow from islets perifused in the presence of extracellular Ca^{2+} and an inhibitory effect of glucose upon ^{45}Ca outflow from islets perifused in the absence of extracellular Ca^{2+} was seen when the release of Ca from the vacuolar system depended on the islet cyclic AMP content (Fig. 4). In Fig. 4, the release of Ca from the vacuolar system was ruled by Eq. 4. A comparable picture was seen with Eq. 3, except that the magnitude of the glucose-induced increase in ^{45}Ca outflow was less pronounced than that illustrated in Fig. 4 (data not shown).

Fig. 5 illustrates the pattern of both ^{45}Ca outflow and insulin release in the stratified model, with discontinuity in the stimulant action of glucose upon Ca inflow into the islet cells. Several features of islet function are adequately simulated, at least from the qualitative standpoint. Such is the case for the biphasic pattern of glucose-induced insulin release (Fig. 5, lower left panel), its supression in calcium-deprived islets (Fig. 5, lower right panel), the biphasic pattern of glucose-stimulated ^{45}Ca outflow from islets perifused in the presence of extracellular Ca^{2+} (Fig. 5, upper left panel), and the monophasic and sustained inhibitory effect of glucose upon ^{45}Ca outflow from islets perifused in the absence of extracellular Ca^{2+} (Fig. 5, upper right panel). The only obvious anomaly in the response to glucose is the absence of an initial fall in ^{45}Ca outflow in response to glucose administration to islets perifused in the presence of extracellular Ca^{2+}.

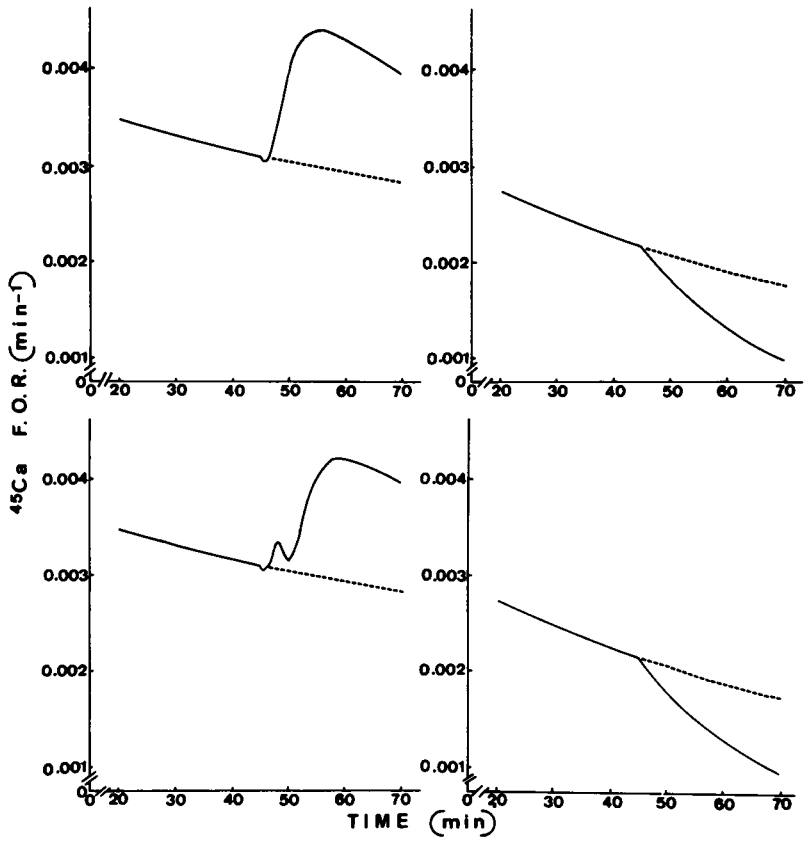

FIGURE 4 Time course for ^{45}Ca fractional outflow rate from islets preincubated for 60 min in the presence of glucose (16.7 mM) and ^{45}Ca (1.0 mM) and perifused from time 0 to 45 min in the absence of glucose and from the 45 min onwards in the presence of glucose (16.7 mM). The perifusive either contained $^{40}Ca^{2+}$ (1.0 mM; *left*) or was deprived of Ca^{2+} (*right*). The results refer to a stratified model in which the peripheral cytosolic (or vacuolar) pool represented 5% of the total cytosolic (or vacuolar) pool. The fractional outflow rate of cytosolic Ca from the inner to outer pool amounted to 0.007 min^{-1}. Glucose, in addition to increasing the inflow rate of Ca^{2+} from 0.120 to 0.275 pmol/islet per min, augmented the fractional removal rate of cytosolic Ca by the vacuolar system from 0.0097 to 0.04 min^{-1}. The fractional rate of Ca release by the vacuolar system was ruled by Eq. 4. The effect of glucose upon Ca inflow into the islets was either sustained (*upper*) or discontinued during the third and fourth minute of exposure to the sugar (*lower*). The dashed lines (---) refer to control experiments performed throughout the 70 min of perifusion in the absence of glucose. F.O.R. stands for the fractional outflow rate.

The model illustrated in Fig. 5 also proved adequate in simulating the stimulant action of glucose upon ^{45}Ca net uptake, cyclic AMP production, and insulin release in islets incubated for 90 min in the absence or presence of glucose (Table II). The true Ca content of the various subcellular pools, whether after 90-min incubation or under steady state conditions, is shown in Table III.

DISCUSSION

The present work relates to an essential and highly controversial aspect of the cationic response of pancreatic islets to glucose. The question under consideration is whether glucose decreases ^{45}Ca outflow from prelabeled and perifused islets by inhibiting a modality of Ca transport across the plasma membrane from the cytosol into the extracellular fluid or by stimulating the uptake of cytosolic Ca by intracellular organelles.

In our previous studies, we have always favored the first of these two mechanisms (5, 11). The present study, however, is based on the alternative hypothesis. Indeed, throughout the present study it is postulated that glucose, in addition to facilitating the entry of Ca into the islet cells, increases the removal rate of cytosolic Ca by the vacuolar system (Fig. 6). The significance of other attributes of our model such as the stratification of the cytosolic and vacuolar Ca pools (2), the regulation of cyclic AMP synthesis by the outer cytosolic Ca concentration (1), and the discontinuity of the stimulant action of glucose upon Ca influx (2, Scholler, Y., V. De Maertelaer, and W. J. Malaisse, manuscript submitted for publication) were fully discussed in earlier publications. Note that in the present model it is the Ca concentration of the outer cytosolic layer that regulates the release of insulin (2).

In our opinion, three novel and important pieces of information are provided by this study. First, it is clearly documented that an increase in vacuolar Ca uptake, sufficiently marked to simulate the inhibitory effect of glucose upon ^{45}Ca outflow, does not necessarily eliminate

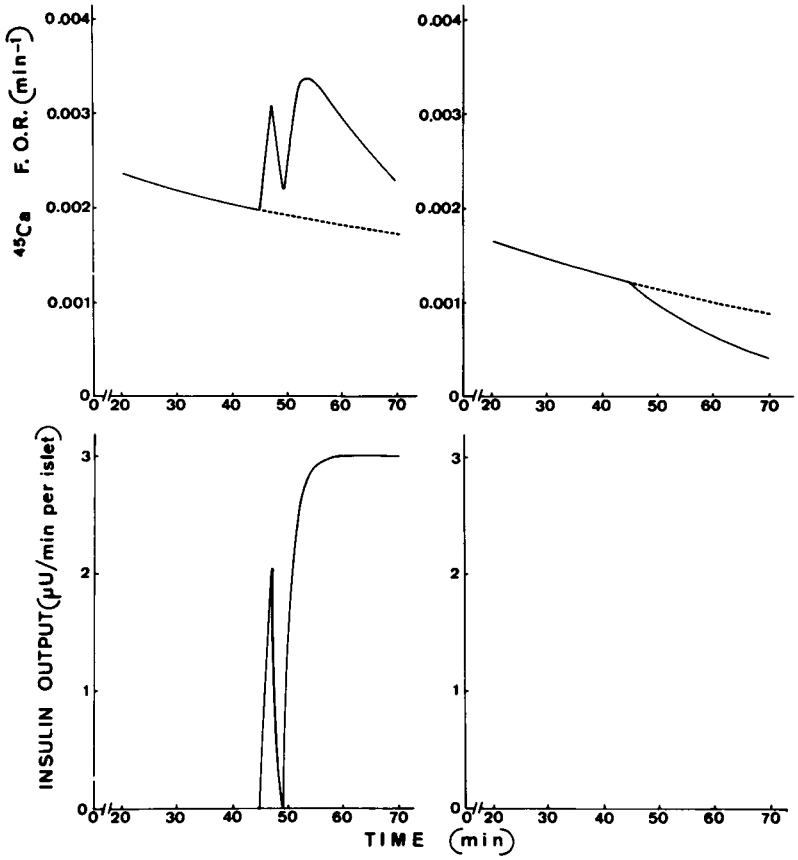

FIGURE 5 Time course for ^{45}Ca fractional outflow rate (*upper*) and insulin release (*lower*) from islets preincubated for 60 min in the presence of glucose (16.7 mM) and ^{45}Ca (1.0 mM) and perifused from time 0 to 45 min in the absence of glucose and from the 45 min onwards in the presence of glucose (16.7 mM). The perfusive either contained $^{40}Ca^{2+}$ (1.0 mM; *left*) or was deprived of Ca^{2+} (*right*). The results refer to the same model as that defined in Fig. 3 (middle *right*), except that the effect of glucose upon Ca inflow into the islets was discontinued during the third and fourth minute of exposure to the sugar. The dashed lines (---) refer to control experiments performed throughout the 70 min of perfusion in the absence of glucose.

TABLE II

^{45}Ca DISTRIBUTION, CYCLIC AMP CONTENT, AND INSULIN RELEASE IN ISLETS INCUBATED FOR 90 MIN IN THE ABSENCE OR PRESENCE OF GLUCOSE (16.7 mM)

	No glucose	16.7 mM glucose
^{45}Ca outer cytosolic pool*	0.1180	0.2642
^{45}Ca inner cytosolic pool*	0.7665	0.7645
^{45}Ca total cytosolic pool*	0.8845	1.0287
^{45}Ca outer vacuolar pool*	0.0762	0.3502
^{45}Ca inner vacuolar pool*	0.3207	1.9588
^{45}Ca total vacuolar pool*	0.3969	2.3090
^{45}Ca cellular pool*	1.2814	3.3377
Cyclic AMP‡	7.80	14.13
Insulin release§	0.00	268.17

*Results are expressed as pmol/islet.
‡Results are expressed as fmol/islet.
§Results are expressed as μU/islet per 90 min.

the capacity of glucose to increase rapidly the cytosolic Ca concentration and to provoke a rapid secretory response.

Second, our present model eliminates one of the unsatisfactory features encountered when glucose is postulated to exert a primary inhibitory action upon Ca transport from

TABLE III

Ca DISTRIBUTION IN ISLETS DEPRIVED OF GLUCOSE (STEADY STATE VALUES) OR EXPOSED TO 16.7 mM GLUCOSE (VALUES AFTER 90-MIN INCUBATION AND STEADY STATE VALUES)*

	No glucose	16.7 mM glucose	
	Steady state	90 min	Steady state
Outer cytosolic pool	0.1299	0.2660	0.2976
Inner cytosolic pool	2.4675	0.9457	5.6548
Total cytosolic pool	2.5974	1.2117	5.9524
Outer vacuolar pool	0.1938	0.3730	0.3488
Inner vacuolar pool	3.6823	7.5361	6.6273
Total vacuolar pool	3.8761	7.9091	6.9761
Cellular pool	6.4735	9.1208	12.9285

*All results are expressed as pmol/islet.

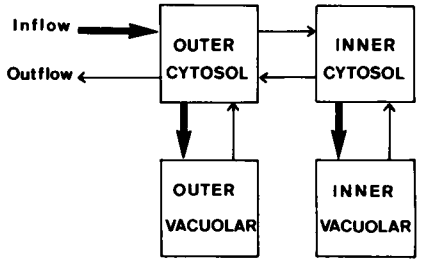

FIGURE 6 Schematic view of Ca handling by the pancreatic β-cell. The thick arrows indicate that glucose stimulates the entry of Ca into the cell and increases the removal of Ca by the vacuolar system, as postulated in the present study.

the cytosol to the extracellular milieu. In the latter case, a sizeable reascension in ^{45}Ca fractional outflow rate is recorded shortly after the initial fall in effluent radioactivity seen in response to glucose adminstration to Ca^{2+}-deprived islets, provided that an allowance is made for the process of Ca-stimulated Ca release by the vacuolar system (Scholler, Y., V. De Maertelaer, and W. J. Malaisse, manuscript submitted for publication). Such a reascension is secondary to the increase in cytosolic Ca concentration, which itself results from the inhibition of Ca outwards transport. In the present model, however, glucose does not exert any primary effect upon Ca outwards transport and, hence, fails to cause a reascension in effluent radioactivity from Ca^{2+}-deprived islets.

The third improvement provided by this study is that, despite the direct or indirect stimulatory effect of cytosolic Ca upon Ca release by the vacuolar system, glucose increases the Ca content of such a system whether judged by radioisotopic (Table II) or nonradioisotopic criteria (Table III). This is consistent with ultrastructural findings (12), but in sharp contrast to the situation found when glucose is postulated to decrease ^{45}Ca fractional outflow rate by inhibiting the efficiency of the transport system responsible for Ca efflux from the β-cell. In the latter situation, glucose slightly increases the ^{45}Ca content of the vacuolar pool, as measured after 90-min incubation, but dramatically lowers the true Ca content of the vacuolar system (Scholler, Y., V. De Maertelaer, and W. J. Malaisse, manuscript submitted for publication). In other words, in the present model as distinct from that defined in the preceding report in this series (Scholler, Y., V. De Maertelaeer, and W. J. Malaisse, manuscript submitted for publication), the stimulatory effect of glucose upon Ca uptake by the vacuolar system prevails, in terms of vacuolar Ca sequestration, over the process of Ca-stimulated Ca release.

In this discussion, we have so far underlined the favorable attributes of our model. However, two unsatisfactory attributes should now be mentioned. On one hand, in the stratified model the absolute value for the fractional outflow rate of ^{45}Ca from the islets was usually below 0.005 min^{-1}, which is less than the physiological value (5). On the other hand, in the presence of extracellular Ca^{2+} glucose failed to cause an initial fall in ^{45}Ca fractional outflow rate, as normally observed before the secondary rise in effluent radioactivity (3, 4). These unsatisfactory features indicate that further sophistication of our model is required to simulate more closely the true behavior of perifused islets. For instance, a glucose-induced initial fall in ^{45}Ca efflux from islets perifused in the presence of extracellular Ca^{2+}, before the secondary rise in effluent radioactivity might be observed if the stimulant action of glucose upon Ca entry were to be initiated somewhat later than the facilitating effect of the sugar upon Ca removal by the vacuolar system. This is not an unrealistic proposal. Indeed, as already mentioned in the Introduction, the stimulation of Ca entry into the β-cell may be secondary to the facilitated uptake of Ca by the vacuolar system; the two phenomena are interconnected through (*a*) a fall in cytosolic Ca concentration, (*b*) the subsequent inactivation of Ca^{2+}-sensitive K^+ channels and the resulting depolarization of the plasma membrane, and (*c*) the eventual gating of voltage-sensitive Ca^{2+} channels.

Two last remarks deserve attention. First, it is shown here that the process of Ca-stimulated Ca release by the vacuolar system, recently introduced in this model (Scholler, Y., V. De Maertelaer, and W. J. Malaisse, manuscript submitted for publication), can be simulated if it is postulated that the release of Ca by the vacuolar system is increased by cyclic AMP. In other words, an increase in the cytosolic Ca concentration could augment the fractional rate of Ca release by the vacuolar system either in a direct manner, as implied by Eqs. 1 and 2, or in an indirect manner through changes in the rate of cyclic AMP synthesis, as implied by Eqs. 3 and 4. Second, we emphasized here that a glucose-induced stimulation of Ca removal by the vacuolar system seems quite compatible with several attributes of islet function. In certain respects, it even appeared as a more satisfactory explanation for the glucose-induced decrease in ^{45}Ca outflow than the alternative hypothesis, namely, the inhibition of a transport system responsible for Ca exit from the islet cells. None of these considerations rules out, however, that the cationic response to glucose, in terms of inhibition of ^{45}Ca outflow, reflects a dual change in both Ca removal by the vacuolar system and the efficiency of Ca outwards transport across the plasma membrane.

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