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Influence of a Tumor-Promoting Phorbol Ester on the Electrical Response of B-cells to Glucose and Glyburide

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SUMMARY

The tumor-promoting phorbol ester, 12-0-tetradecanoylphorbol-13-acetate (TPA), enhances the secretory responses of pancreatic islet cells to glucose and a hypoglycemic sulfonylurea. The influence of TPA on the electrical activity induced by glucose or the sulfonylurea, glyburide, was assessed to determine whether TPA altered the conductances of the K^+ and Ca^{2+} channels subserving depolarization and spike generation. TPA, 0.2 μM, did not alter the membrane potential obtained with 0–5.6 mM glucose. With 7.0, 8.4, and 11.0 mM glucose, TPA increased the duration of the active phase of oscillatory spike activity more than 2-fold. Upon withdrawal of glucose or glucose plus TPA, the cells hyperpolarized and spike activity ceased. In cells not exposed to TPA, the subsequent addition of 10 nM glyburide resulted in slow depolarization after 10–13 min and occurred at a rate of 1.2 ± 0.2 mV/min. In cells preexposed to TPA, depolarization commenced by 4–8 min and occurred at a rate of 3.2 ± 0.4 mV/min. The magnitude of depolarization was 15–20 mV with both conditions. Small amplitude spikes appeared during depolarization. After depolarization, the onset of high amplitude spikes appeared sooner in TPA-treated B-cells and occurred 2.6 times more frequently during the first 6.5 min after depolarization than in B-cells not exposed to TPA. A stable pattern of spike generation was achieved twice as fast in TPA-treated cells. TPA enhancement of glucose- and glyburide-induced spike activity suggests that the insulinotropic action of TPA is mediated by augmenting the influx of Ca^{2+} into the B-cell via voltage-sensitive Ca^{2+} channels.

INTRODUCTION

The mechanism by which insulinotropic agents elicit an electrical response from B-cells is generally considered to be via a decrease in K^+ permeability leading to depolarization and a consequent activation of voltage-dependent Ca^{2+} channels. This is the case for both glucose and hypoglycemic sulfonylureas (1–4). However, concentrations of glucose within the range of 7.0–11.1 mM generate oscillations of the membrane potential with bursts of spikes elicited from the depolarized phase, whereas hypoglycemic sulfonylureas elicit depolarization and constant spike activity (2). Although the action of glucose on these biophysical events may be mediated by factors associated with glucose metabolism (5), it is of interest that the action of a sulfonylurea may occur directly by accumulation in the plasma membrane, where it may participate in facilitated Ca^{2+} uptake into the B-cell (6) or directly initiate biophysical mechanisms controlling Ca^{2+} and K^+ conductances (3, 4, 7).

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It has been reported that the tumor-promoting phorbol ester, TPA,[1] profoundly enhances the secretory response of islets to glucose or the sulfonylurea, glicazide, with no effect on $^{86}Rb^+$ or $^{45}Ca^{2+}$ uptake (8–10). Nevertheless, it was suggested that TPA may interact with glicazide at the plasma membrane and facilitate its action as a Ca^{2+} ionophore via a voltage-independent pathway. A series of studies has further indicated that TPA activates a Ca^{2+}-activated phospholipid-dependent protein kinase (protein kinase C) obtained from rat brain to which it also binds (11). Furthermore, TPA substitutes for unsaturated diacylglycerol, which is generated by phosphatidylinositol turnover in response to physiological stimuli (11–13). Although diacylglycerol-activated protein kinase C has been demonstrated to exist in islets (14), an obvious gap exists in our knowledge of the role of this enzyme in stimulation of plasma membrane events associated with insulin release.

Determination of the influence of TPA on electrical activity induced by glyburide is a necessary prerequisite

[1] The abbreviation used is: TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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for assessing not only the synergism between TPA and a sulfonylurea, but to gain insight into the role of TPA in mediating insulin release via alteration of biophysical phenomena controlling Ca^{2+} permeability of the plasma membrane. Our results indicate that TPA augments glucose-induced electrical activity and markedly enhances both the dynamics and magnitude of the electrical response to glyburide.

METHODS

Islets were partially microdissected from the pancreas of a CBA/J male retired-breeder mouse (Jackson Laboratories, Bar Harbor, Mass.). The islets were perifused in a chamber at a flow rate of 2 ml/min with Krebs-Ringer bicarbonate buffer with the addition of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (15). The medium was maintained at 37° and pH 7.4 by equilibration with 95% O_2 and 5% CO_2. Change of solution within the chamber was accomplished by less than 5 sec. Additions were made to the medium immediately before use in an experiment. Standard electrophysiological procedures were used (15). TPA (Sigma Chemical Company, St. Louis, Mo.) was dissolved in ethanol with final dilution to 0.1% ethanol for all concentrations tested. Glyburide (gift from The Upjohn Company, Kalamazoo, Mich.) was dissolved in dimethyl sulfoxide and diluted to 0.01% for all concentrations tested. The statistical significance of differences between groups was determined by Student's *t*-test for paired or unpaired data.

RESULTS

The experimental protocol was designed to test the influence of TPA on the electrical response of each B-cell to glucose and glyburide. The cell was impaled in 11.1 or 16.7 mM glucose to identify an electrically active B-cell after which the solution was changed to one containing 0–7.0 mM glucose. The subsequent addition of TPA did not influence the membrane potential obtained in the presence of 0–5.6 mM glucose (data not shown). Oscillatory activity appeared 3–4 min after the addition of 7.0 mM glucose (Fig. 1). After about 7 min, 0.1% ethanol (control) or 0.2 μM TPA was added. The initial burst of activity and quiescent period was found to be due to the 0.1% ethanol and was not a switching artifact, as determined by changing the solutions to 7.0 mM glucose without ethanol. It is of interest that ethanol did not elicit this response at subthreshold levels of glucose. Glucose was then increased to 8.4 and 11.1 mM in the presence of 0.1% ethanol or 0.2 μM TPA (Fig. 1; Table 1). When the glucose concentration was increased, there was an initial period during which the bursts were prolonged. This is typical of the biphasic electrical response to glucose characterized by initial bursts of prolonged active phases, after which bursts of shorter duration appear. The electrical response was calculated as the percentage of time occupied by the active phase of all bursts generated 2 min after changing solutions divided by the duration of the active plus silent phases during the same period. The cells were exposed to each glucose concentration for 5–10 min. TPA significantly augmented the duration of the active phase more than 2-fold for each concentration of glucose (Table 1).

After exposure of the cells to glucose with or without TPA, the solution was changed to one containing no additions (Fig. 2, *Cell 2* and *Cell 3*. The influence of TPA on the glucose-induced electrical response was reversible, since the cell hyperpolarized and oscillatory activity ceased after withdrawal of glucose and TPA (Fig. 1, *Cell 2*; Fig. 2, *Cell 3*). After 15 min, 10 nM glyburide was added. The initial plan was to add glyburide with or without TPA, but this was found not to be necessary since the cells that were preexposed to TPA responded to glyburide more rapidly than did cells exposed only to ethanol.

In initial experiments, 10 μM glyburide in the absence or presence of 2.8 mM glucose was found to initiate rapid depolarization by 20 sec and spikes of similar amplitude, 20–25 mV, by about 60 sec (Fig. 2, *Cell 1*). The spike frequency of five spikes per sec was the highest 1 min after appearance and gradually declined to about two spikes per sec as the duration of the spikes increased from 200 to 450 msec (Fig. 2, *Cell 1*). The concentration of glyburide was reduced to 10 nM in the absence of glucose in an effort to find a concentration eliciting a quantitatively lower electrical response, and it was subsequently found that this concentration elicited an electrical response substantially different from that observed at 10 μM (Fig. 2, *Cell 2*). In control experiments (data not shown), 0.01% dimethyl sulfoxide, the final level obtained after dilution of the glyburide stock solution, was found to have no influence on the membrane potential.

After addition of glyburide, the B-cells began to depolarize slowly by 10–13 min, and the rate of depolarization was 1.2 ± 0.2 mV ($n = 3$). In cells preexposed to TPA, depolarization commenced by 4–8 min, and the rate of depolarization was 3.2 ± 0.4 mV/min ($n = 3$). Although the glyburide-induced rate of depolarization for the cells preexposed to TPA was 2.6 times faster than that for cells exposed to glyburide alone, the magnitude of depolarization under both conditions was about the same and ranged from 15 to 20 mV.

As the cells depolarized, small amplitude spikes were generated. After depolarization, larger amplitude spikes began to appear and increased in frequency, achieving a relatively stable pattern of spike generation after about 25–30 min. In cells preexposed to TPA, the larger amplitude spikes appeared sooner and a stable pattern of spike activity was obtained in less than half the time. The time dependency of this pattern of spike activity in individual cells after depolarization is shown in Fig. 3. The spike frequency was assessed for every 30 sec after the cell depolarized. With TPA preexposure, the appearance of the high amplitude spikes, 6.5 min after depolarization, was 2.6 times greater than that obtained in cells stimulated with glyburide alone.

DISCUSSION

TPA was found to augment electrical activity induced by either glucose or glyburide. However, TPA in the absence of a stimulant or in the presence of a level of glucose that did not elicit oscillatory activity had no influence on the membrane potential. These results differ from the observation that TPA, in the absence of glucose, elicits a slow onset of insulin release which is sustained and not rapidly reversible (9). The present observations suggest that TPA stimulates insulin release not via the conductive uptake of Ca^{2+} into the cell via spike activity,

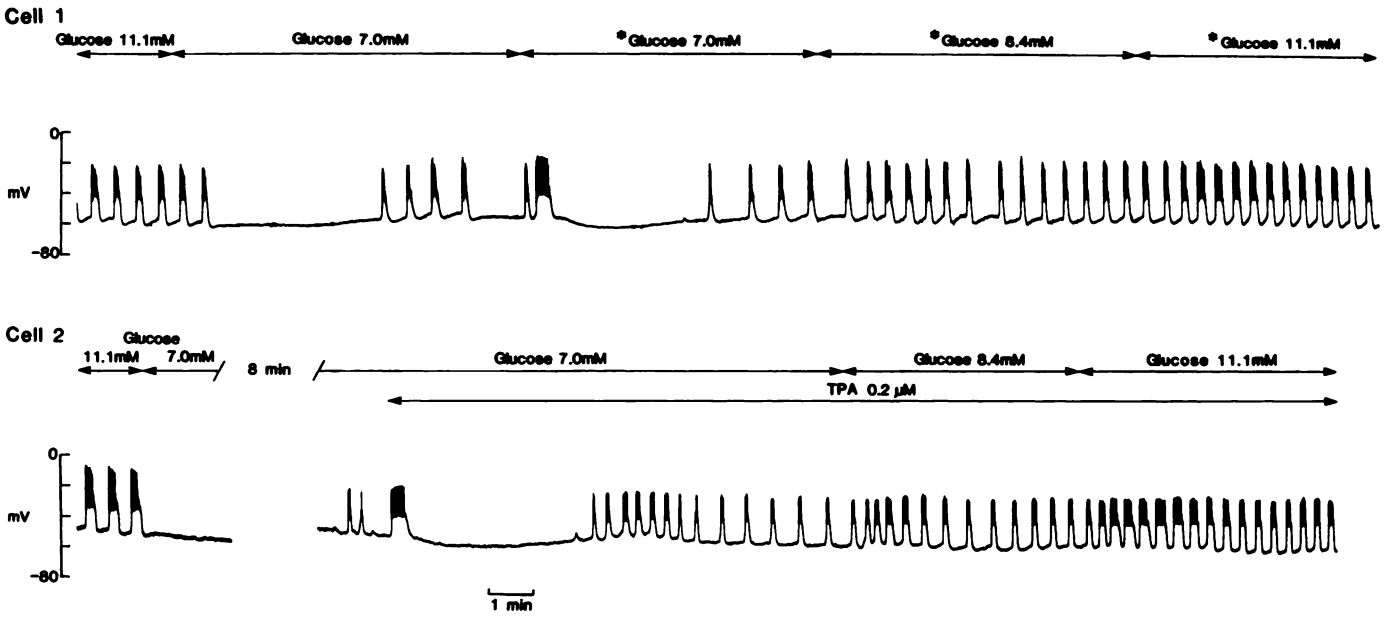

FIG. 1. *Influence of TPA on glucose-induced electrical activity*

The B-cells were impaled in 11.1 mM glucose, after which the solution was changed to one containing 7.0 mM glucose. An *asterisk* indicates that ethanol, 0.1%, was added to Cell 1 for control. The burst of spike activity, which was also observed upon addition to Cell 2 of 0.2 μM TPA from a stock solution dissolved in ethanol, was not due to switching solutions to one containing the same level of glucose (data not shown). The percentage duration of the active phase was quantitated for each concentration of glucose (see Table 1).

TABLE 1

Influence of TPA on glucose-induced electrical response

After impaling an islet cell in 11.1 mM glucose, the solution was changed to one containing 7.0 mM glucose. For solutions containing only glucose, 0.1% ethanol was then added after the electrical response to 7.0 mM glucose had stabilized. The stock solution of TPA in ethanol was added to each glucose solution to obtain a 0.2 μM concentration with a dilution of ethanol to 0.1%. The *p* value denoting the statistical signficance of B as compared with A is <0.05 for each concentration of glucose. Each value of the mean of at least four experiments.

Glucose concentration	% Duration of active phase[a]		B/A
	Glucose (A)	Glucose + TPA (B)	
mM			
7.0	7 ± 2	18 ± 3	2.6
8.4	17 ± 2	35 ± 6	2.2
11.1	34 ± 4	69 ± 1	2.1

[a] Mean ± standard error of the mean.

but by mobilizing Ca^{2+} from intracellular organelles into the cytosol. This analysis is supported by Ca^{2+} flux studies which showed that TPA does not stimulate a net uptake of $^{45}Ca^{2+}$ into the islet cells, but elicits a slow rise in $^{45}Ca^{2+}$ efflux in the absence or presence of extracellular Ca^{2+} (8, 9).

The absence of any effect of TPA on the membrane potential with no glucose or subthreshold levels of glucose is consistent with the observation that TPA has no effect on $^{86}Rb^{+}$ efflux (8, 9). This further indicates that TPA does not induce Ca^{2+} entry into the B-cell indirectly by decreasing the K^{+} permeability and activating voltage-sensitive Ca^{2+} channels. It is generally accepted, based on ion flux and electrical studies, that stimulatory effects of glucose and sulfonylureas are mediated via a reduction in K^{+} permeability leading to depolarization and stimulation of Ca^{2+} influx (1–3, 7).

When glucose is present at a concentration sufficient to elicit oscillatory electrical activity, the addition of TPA augments the electrical response by increasing the duration of the active phase 2-fold. This result indicates that TPA enhances the influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels. TPA does not alter $^{45}Ca^{2+}$ uptake in the absence of glucose, and there is no available evidence to assess the influence of TPA on $^{45}Ca^{2+}$ uptake in the presence of 7.0–11.1 mM glucose (8, 9). Nevertheless, Ca^{2+} net uptake studies may not be sensitive enough to detect the small increment in the cytosolic pool of ionized Ca^{2+} due to TPA-induced conductive entry of Ca^{2+} (9). In addition, TPA does not influence glicazide-induced $^{45}Ca^{2+}$ uptake, but increases to a small extent the influence of glicazide on $^{45}Ca^{2+}$ efflux (8). Since Ca^{2+} efflux is partially due to influx of Ca^{2+} into the islet cell, this finding supports the possibility that TPA interacts with the membrane in a manner that augments the influence of a sulfonylurea on Ca^{2+} transport across the plasma membrane (8). The earlier onset of spike activity and also of large-amplitude spikes in cells preexposed to TPA are consistent with this hypothesis.

It was necessary to use a much lower level of glyburide than has been used previously in electrophysiological studies (16). It has been shown that 20 μM glyburide induces a rapid onset of spike activity. After a few minutes the frequency of spikes dramatically decreases after which the spike frequency stabilizes and persists even after removal of the drug (16). These results are similar to the dynamics of the secretory response to glyburide (17). We obtained a similar electrical response

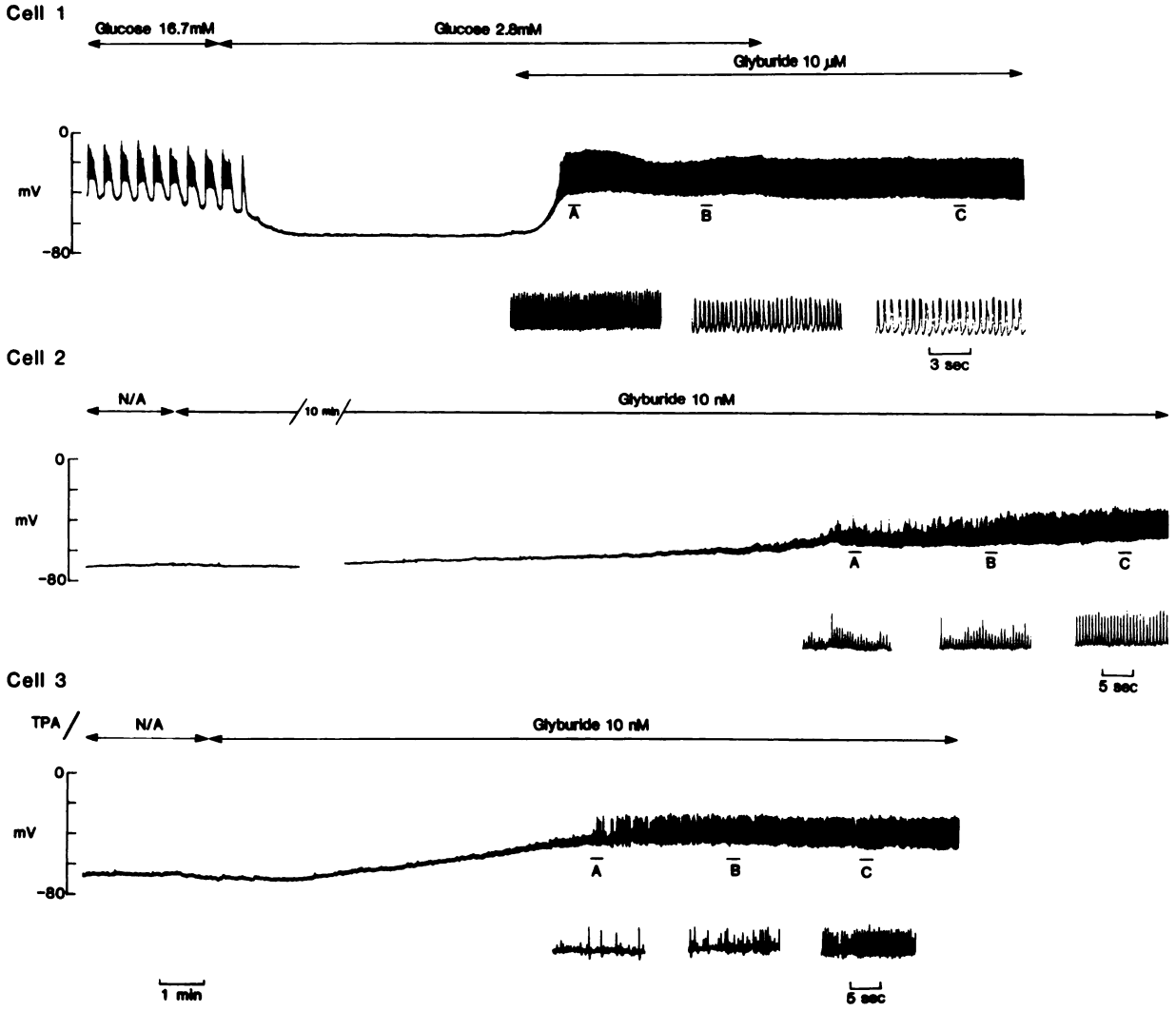

FIG. 2. *Influence of TPA on glyburide-induced electrical activity*

In Cell 1, 10 μM glyburide elicited rapid depolarization and spike activity. The other cells were initially impaled in 11.1 mM glucose and treated as shown in Fig. 1. After the glucose dose-response was obtained in the absence (Cell 2) or presence (Cell 3) of 0.2 μM TPA, a solution containing no additions, *N/A*, was applied. Glyburide, 10 nM, was subsequently added to the solution. Fast record speeds *A*, *B*, and *C* are shown to illustrate the shape and frequency of the spikes at the time intervals designated.

with 10 μM glyburide. Because the addition of TPA to 10 μM glyburide had no discernible effect, it was necessary to use a concentration that elicited a less intense electrical response, as was subsequently obtained with 10 nM glyburide. The dynamics of the electrical response was substantially different. First, there was slow depolarization that did not even appear until at least 10 min after addition of glyburide. The spikes were initiated as the cells depolarized, but higher-amplitude spikes appeared after depolarization to a stable potential. A relatively constant pattern of spike activity was achieved 25–30 min after glyburide addition. Preexposure to TPA substantially decreased the lag time before depolarization and spike activity commenced.

It is difficult to compare directly the synergistic effect obtained between TPA and glyburide on the electrical response of mouse islets with that found with TPA and glicazide on the secretory response of rat islets (8). Besides the use of different sulfonylureas, different concentrations of glyburide (10 nM) and glicazide (3.1 and 62 μM) were used in the electrical and secretory experiments, respectively. Glicazide at 3.1 μM in the absence of glucose failed to stimulate insulin release even when added simultaneously with 0.2 μM TPA. However, addition of 3.1 μM glicazide to islets already exposed for 44 min to TPA elicited a rapid and sustained increase in insulin output (8). These results indicate that TPA lowered the threshold concentration necessary for obtaining a secretory response to the sulfonylurea. The electrical response to cells preexposed to TPA also indicates that the phorbol ester augments the electrical response obtained with glyburide. However, although TPA decreased the time lag before glyburide induced depolarization, the change in the membrane potential was not different from

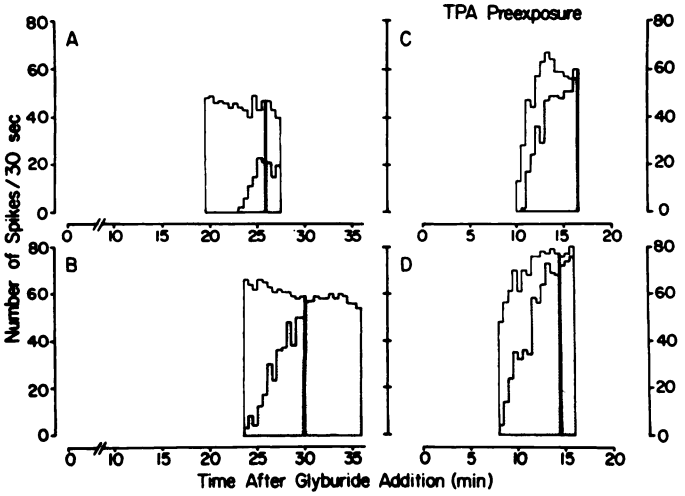

FIG. 3. *Time-related pattern of spike frequency in response to TPA in B-cells stimulated with glyburide*

The graphs shown in A and B were obtained from the electrical responses of B-cells to 10 nM glyburide; in C and D the data were obtained from cells preexposed to 0.2 μM TPA as described in the legend to Fig. 2. The spike frequency for each 30-sec interval was calculated after the cells depolarized. The *open areas* represent the total spike frequency and the *shaded areas* represent the pattern of generation of high-amplitude spikes. These spikes were at least 2 times higher than the smaller-amplitude spikes. For quantitation, the areas under the curves were calculated for the first 6.5 min after depolarization, as indicated by the vertical lines, to determine the proportion of high-amplitude spikes related to total spikes generated within this period.

that obtained with glyburide alone. It seems logical that TPA may alter the setpoint for alteration of the active state of K^+ and Ca^{2+} channels so that they are more sensitive to the subsequent addition of glyburide.

It is interesting to speculate that TPA may directly activate voltage-sensitive Ca^{2+} channels by alteration of the phospholipid domain surrounding the Ca^{2+} channel or may do so indirectly by activation of protein kinase C at a site specific for diacylglycerol, a breakdown product of polyphosphatidylinositol or phosphatidylinositol (11–13, 18). Phosphorylation of a protein controlling Ca^{2+} channel permeability may subsequently lead to an enhancement of the influence of glucose or glyburide on this channel.

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