Protein Kinase C Modulates the Insulin Secretory Process by Maintaining a Proper Function of the β-Cell Voltage-activated Ca\(^{2+}\) Channels*

(Received for publication, August 19, 1993, and in revised form, September 21, 1993)

Per Arkhammar†, Lisa Juntti-Berggren‡, Olof Larsson§, Michael Welsh¶, Ewa Nänberg¶, Åke Sjöholm†, Martin Köhler∥, and Per-Olof Berggren‡∥

From the †Department of Endocrinology, Karolinska Institute, The Rolf Luft Center for Diabetes Research, Box 60500, Karolinska Hospital, S-104 01 Stockholm, Sweden, the ‡Department of Medical Cell Biology, Biomedical Center, Uppsala University, Box 571, S-751 23 Uppsala, Sweden, and the §Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden

In the present study an attempt was made to further elucidate the molecular mechanisms whereby protein kinase C (PKC) modulates the β-cell stimulus-secretion coupling. Regulation of Ca\(^{2+}\) channel activity, [Ca\(^{2+}\)]\(_{\text{I}}\), and insulin release were investigated in both normal pancreatic mouse β-cells and in similar β-cells deprived of PKC activity. [Ca\(^{2+}\)]\(_{\text{I}}\) was measured with the intracellular fluorescent Ca\(^{2+}\) indicator fura-2 and the Ca\(^{2+}\) channel activity was estimated by the whole cell configuration of the patch-clamp technique. To reveal the various isoenzymes of PKC present in the mouse β-cell, proteins were separated by one-dimensional gel electrophoresis and Western blotting was performed. The production of inositol phosphates was measured by ion-exchange chromatography and insulin release was measured radioimmunologically. Acute stimulation with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate resulted in suppression of both the carbamylcholine-induced increase in [Ca\(^{2+}\)]\(_{\text{I}}\), and production of inositol 1,4,5-trisphosphate. Under these conditions the increase in [Ca\(^{2+}\)]\(_{\text{I}}\) in response to glucose was similar to that found in control cells. When β-cells were deprived of PKC, by exposure to 200 μM 12-O-tetradecanoylphorbol-13-acetate for 24–48 h, there was an enhanced response to carbamylcholine. This response constituted increases in both the [Ca\(^{2+}\)]\(_{\text{I}}\) signal and production of inositol 1,4,5-trisphosphate. Interestingly, cells with down-regulated PKC activity responded more slowly to glucose stimulation, when comparing the initial increase in [Ca\(^{2+}\)]\(_{\text{I}}\), than control cells. On the other hand, the maximal increase in [Ca\(^{2+}\)]\(_{\text{I}}\) was similar whether or not PKC was present. Moreover, PKC down-regulated cells exhibited a significant reduction of maximal whole cell Ca\(^{2+}\) currents, a finding that may explain the altered kinetics with regard to the [Ca\(^{2+}\)]\(_{\text{I}}\), increase in response to the sugar. Both the α and β\(_{1}\) forms of the PKC isoenzymes were present in the mouse β-cell and were also subjected to PKC down-regulation. Hence, either of these isoenzymes or both may be involved in the modulation of phospholipase C and Ca\(^{2+}\) channel activity. Since insulin release under physiological conditions is critically dependent on Ca\(^{2+}\)-influx through the voltage-gated L-type Ca\(^{2+}\) channels, the kinetics of hormone release was expected to demonstrate a similar delay as that of the [Ca\(^{2+}\)]\(_{\text{I}}\), increase. Although not as pronounced, such a delay was indeed also observed in the onset of insulin release. There was, however, no effect on the total amounts of hormone released. The present study confirms that PKC has multiple roles and thereby interacts at different sites in the complex series of events constituting the β-cell signal-transduction pathway. It is suggested that PKC may be tonically active and effective in the maintenance of the phosphorylation state of the voltage-gated L-type Ca\(^{2+}\) channel, enabling an appropriate function of this channel in the insulin secretory process.

The ultimate response of the pancreatic β-cell to a number of different stimuli, such as glucose and acetylcholine, is insulin release into the bloodstream. This response is the result of integration and coordination of several plasma membrane and intracellular processes and involves changes in activity of ion channels, membrane potential, [Ca\(^{2+}\)]\(_{\text{I}}\), intracellular levels of cyclic nucleotides and phosphoinositide turnover (1–4). The latter process is due to activation of PLC, generating InsP\(_3\) and DAG (5). InsP\(_3\) releases intracellularly bound Ca\(^{2+}\) (6) and DAG activates PKC (7). This enzyme can also be directly activated by nanomolar concentrations of the phorbol ester TPA (7).

In normal pancreatic β-cells, activation of PKC with TPA increases insulin release despite no change or a lowering in [Ca\(^{2+}\)]\(_{\text{I}}\), (8). Addition of TPA does not interfere with either intracellular buffering of [Ca\(^{2+}\)]\(_{\text{I}}\), or the ability of InsP\(_3\) to release Ca\(^{2+}\) from internal stores (8). In the insulin-producing RINm5F tumor cells, PKC activation with TPA has been reported to increase insulin release and lower [Ca\(^{2+}\)]\(_{\text{I}}\) (9, 10). The lowering in [Ca\(^{2+}\)]\(_{\text{I}}\), may be explained by an active export of Ca\(^{2+}\) to the extracellular space (11), a mechanism known to operate also in other cell types (12, 13). In RINm5F cells it has also been reported that TPA induces an increase in [Ca\(^{2+}\)]\(_{\text{I}}\), due to membrane depolarization (14, 15). The depolarization is suggested

* This study was supported by grants from the Swedish Medical Research Council (03X-09890, 04X-09891, 13X-09880, 12X-09884), the Bank of Sweden Tercentenary Foundation, the Swedish Diabetes Association, Funds of the Karolinska Institute, the Swedish Society for Medicine, Novo Industry, the Nordic Insulin Foundation, the Clas Groschinsky Memorial Foundation, the Helge Axelson Johnson Foundation, the Royal Swedish Academy of Sciences, Lars Hiertas Memorial Foundation, Sven and Ingrid Thurfings Foundation, Magnus Bergvalls Foundation, and Ulf Widengrens Memorial Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

∥ To whom correspondence should be sent. Tel.: 46-8-729-5731; Fax: 46-8-303-458.
PKC Activation and Down-regulation—Activation and down-regulation of PKC were achieved by incubation of cells in the presence of 200 nM TPA. Although the effect of carbamylcholine was significant, the carbamylcholine-induced increase in [Ca\(^{2+}\)]\(_i\) in the presence of 10 nM TPA was not as pronounced as in control cells (middle panel of Fig. 1A). In the presence of 10 nM TPA, 100 μM carbamylcholine still increased [Ca\(^{2+}\)]\(_i\), reflecting InsP\(_2\)-mediated release of Ca\(^{2+}\) from intracellular stores (28). The middle panel of Fig. 1A shows the effects on [Ca\(^{2+}\)]\(_i\) in the presence of 10 nM TPA. Although the effect of carbamylcholine was significantly reduced, the increase in [Ca\(^{2+}\)]\(_i\), in response to glucose was similar to that found in control cells (middle and right panels of Fig. 1A). This acute effect of TPA on the carbamylcholine-induced increase in [Ca\(^{2+}\)]\(_i\) has previously been demonstrated in both RINm5F cells (39) and normal β-cells (8). It was suggested that, as for other cell types, the effect was due to inhibition of InsP\(_3\)-mediated release of Ca\(^{2+}\) from intracellular stores (28). The middle panel of Fig. 1A shows the effects on [Ca\(^{2+}\)]\(_i\) in the presence of 10 nM TPA. Although the effect of carbamylcholine was significantly reduced, the increase in [Ca\(^{2+}\)]\(_i\), in response to glucose was similar to that found in control cells (middle and right panels of Fig. 1A). This acute effect of TPA on the carbamylcholine-induced increase in [Ca\(^{2+}\)]\(_i\) has previously been demonstrated in both RINm5F cells (39) and normal β-cells (8). It was suggested that, as for other cell types, the effect was due to inhibition of InsP\(_3\)-mediated release of Ca\(^{2+}\) from intracellular stores (28). The middle panel of Fig. 1A shows the effects on [Ca\(^{2+}\)]\(_i\) in the presence of 10 nM TPA. Although the effect of carbamylcholine was significantly reduced, the increase in [Ca\(^{2+}\)]\(_i\), in response to glucose was similar to that found in control cells (middle and right panels of Fig. 1A). This acute effect of TPA on the carbamylcholine-induced increase in [Ca\(^{2+}\)]\(_i\) has previously been demonstrated in both RINm5F cells (39) and normal β-cells (8). It was suggested that, as for other cell types, the effect was due to inhibition of InsP\(_3\)-mediated release of Ca\(^{2+}\) from intracellular stores (28).
Fig. 1. Effects of glucose, D-600, and carbamylcholine on [Ca^{2+}]_{i} in fura-2 loaded monolayers of pancreatic β-cells. A: left panel, control cells; middle panel, 10 nM TPA present throughout the experiment; right panel, average of acute increases in [Ca^{2+}]_{i} measured as the increase in 340/380 ratio in response to glucose (left) and carbamylcholine (right), as added in the previous panels. Mean values ± S.E. for four to five experiments. *, p < 0.05 (Student’s unpaired t test). B: left panel, control cells; middle panel, cells treated with 200 nM TPA for 48 h; right panel, average of acute increases in [Ca^{2+}]_{i}, measured as the increase in 340/380 ratio in response to glucose (left) and carbamylcholine (right), as added in the previous panels. Mean values ± S.E. for nine to ten experiments. *, p < 0.05 (Student’s unpaired t test).

**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Agonist</th>
<th>InsP_{3} synthesis (dpm/μg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me_{6}SO</td>
<td>Cch, 100 μM</td>
<td>3730 ± 710</td>
</tr>
<tr>
<td>10 nM TPA</td>
<td>Cch, 100 μM</td>
<td>4970 ± 550*</td>
</tr>
<tr>
<td>10 nM TPA</td>
<td>Cch, 100 μM</td>
<td>4060 ± 440</td>
</tr>
</tbody>
</table>

*p < 0.05 versus control cells (Student’s paired t test).

Acetic islets (22) and RINm5F cells (39) and reduces that of pancreatic β-cells by more than 80% (8). Subsequent to down-regulation, the sensitivity to TPA was lost, whereas glucose-stimulated increases in [Ca^{2+}]_{i} and insulin release were still present (8, 22). We now demonstrate that, compared to control cells (Fig. 1B, left panel), β-cells with down-regulated PKC activity had a delayed, but of similar magnitude, response to 20 mM glucose (Fig. 1B, right panel). The time lag from stimulation to peak [Ca^{2+}]_{i} levels was 2.96 ± 0.23 min in control cells and 4.65 ± 0.21 min in TPA-treated cells (p < 0.0001, n = 7–8, Student’s unpaired t test), when [Ca^{2+}]_{i} was measured in small aggregates of β-cells in a microscopic system. When similar experiments were conducted on a large number of β-cells on a coverslip in a cuvette in a spectrofluorometer, the time lag from stimulation to peak [Ca^{2+}]_{i} level was 1.83 ± 0.16 min in control cells and 2.69 ± 0.18 min in cells treated with TPA (mean values ± S.E., p < 0.005, n = 14, Student’s unpaired t test). The response to carbamylcholine was significantly larger in the TPA-treated cells (Fig. 1B, middle and right panel). The latter effect was paralleled by an increased production of InsP_{3} and a tendency for increased InsP_{3} production, as shown in Table II. Thus, cells with preserved PKC activity, cultured in the presence of MeSO solvent only, and stimulated with carbamylcholine increased their InsP_{3} formation by 137 ± 14%, as compared to control cells incubated without carbamylcholine. In TPA-treated cells the corresponding value was 194 ± 22% (mean values ± S.E., p < 0.05, n = 6, Student’s unpaired t test). Similar results have been obtained in RINm5F cells with down-regulated PKC activity and stimulated by vasopressin (39).

To elucidate the molecular mechanisms behind the delayed increase in [Ca^{2+}]_{i} in cells deprived of PKC, which may be explained by increased buffering of Ca^{2+} or a slower influx of the ion, whole cell Ca^{2+} currents were analyzed using the patch-clamp technique. In Fig. 2, A–C, it is demonstrated that the Ca^{2+} currents were significantly reduced in cells with down-regulated PKC activity. No difference was observed with respect to the voltage threshold for activation of the Ca^{2+} channels. It was of interest to see if this marked and easily detectable effect was paralleled by a diminished increase in [Ca^{2+}]_{i}, in response to an elevation of the extracellular K^{+} concentration with 25 mm. Such an elevation in K^{+} concentration depolarizes the β-cells to about –10 mV (compare with Fig. 2C). Indeed, (Fig. 2D), the initial increase in [Ca^{2+}]_{i}, in response to 25 mM K^{+} was significantly reduced in β-cells with down-regulated PKC activity. The finding that the pancreatic β-cell Ca^{2+} currents are reduced when PKC is inactive is interesting, since it suggests that PKC maintains a critical degree of phosphorylation...
whole cell configuration of the patch-clamp technique. Currents were recorded during depolarizing voltage steps to membrane potentials between monolayers of pancreatic β-cells, presented as increase in 340:1380 ratio, in response to an increase in the extracellular environment. We revealed the presence of Ca2+ influx in response to the observed effects on the PLC-system as well as the voltage-gated L-type Ca2+ channels (Fig. 3A). Both of these forms were down-regulated by prolonged TPA treatment (Fig. 3A and B, s lanes), observed as a disappearance of the former visible bands (cf. Fig. 3A and B, s lanes). Hence, the present data suggest that both the α and β1 forms of the PKC isoforms may be involved in the modulation of the β-cell stimulus-secretion coupling.

The main concept is that Ca2+-influx through the voltage-gated L-type Ca2+ channels has a decisive role in regulating the insulin secretory response. Hence, the reduced Ca2+ conductance and thereby delayed increase in [Ca2+]i observed in the present study, subsequent to PKC down-regulation, should be expected to result in a retarded release of insulin. Although not pronounced, detailed analysis of the dynamics of peak insulin release from TPA-treated cells (Fig. 4B) indeed revealed a delay of peak insulin values in response to glucose. In the analysis of peak insulin release, the curve for each perifusion experiment, between the 10th (7.2 min) and 36th (15.0 min) values, was fitted to a 6th degree polynomial function. The time point for the maximum value of the polynomial function was then used to obtain the time lags from stimulation with glucose to peak insulin release. For curve fitting we used the Marquardt-Levenberg algorithm within the program Sigma Plot version 4.10 from Jandel Corp. Mean value ± S.E. for the difference in time, from glucose stimulation to peak insulin release, between con-

### Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Agonist</th>
<th>Inositol phosphate synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>InsP1</td>
</tr>
<tr>
<td>15 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me2SO</td>
<td>Cch, 100μM</td>
<td>39,300 ± 12,200</td>
</tr>
<tr>
<td>Me2SO</td>
<td>Cch, 100μM</td>
<td>44,600 ± 14,900</td>
</tr>
<tr>
<td>200 nm TPA</td>
<td>Cch, 100μM</td>
<td>32,700 ± 12,200</td>
</tr>
<tr>
<td>48 h</td>
<td>Cch, 100μM</td>
<td>41,000 ± 19,600</td>
</tr>
<tr>
<td>Me2SO</td>
<td>Cch, 100μM</td>
<td>28,600 ± 7,760</td>
</tr>
<tr>
<td>Me2SO</td>
<td>Cch, 100μM</td>
<td>33,300 ± 9,430</td>
</tr>
<tr>
<td>200 nm TPA</td>
<td>Cch, 100μM</td>
<td>31,800 ± 8,490</td>
</tr>
<tr>
<td>200 nm TPA</td>
<td>Cch, 100μM</td>
<td>37,700 ± 14,200</td>
</tr>
</tbody>
</table>

* p < 0.05 versus control cells.

b p < 0.01 versus control cells (Student’s paired t test).
Protein Kinase C and the Insulin Secretory Process

The acute effect of PKC stimulation on Ca\textsuperscript{2+} handling has been studied in both insulin producing RINm5F cells (9–11, 14, 15, 39) and normal pancreatic β-cells (8). In RINm5F cells, activation of PKC by TPA reduced both basal (9, 11) and elevated [Ca\textsuperscript{2+}l] in response to K\textsuperscript{+} depolarization (9, 10). The effect at elevated [Ca\textsuperscript{2+}l] was suggested to reflect closure of voltage-activated Ca\textsuperscript{2+} channels (10). However, this hypothesis was not supported by direct electrophysiological measurements of whole cell Ca\textsuperscript{2+} currents in RINm5F cells, which were actually found to increase upon acute TPA stimulation (50). Furthermore, using Ba\textsuperscript{2+} as charge carrier and thereby a Ca\textsuperscript{2+} analogue, it has been demonstrated that stimulation of RINm5F cells with glyceraldehyde results in an increase in the magnitude of Ca\textsuperscript{2+} currents through individual Ca\textsuperscript{2+} channels (51). Glyceraldehyde is known to activate PKC in these cells (14) and part of its property as an insulin secretagogue has been attributed to PKC-mediated closure of ATP-regulated K\textsuperscript{+} channels, leading to membrane depolarization and thereby influx of Ca\textsuperscript{2+} through the voltage-activated L-type Ca\textsuperscript{2+} channels (14, 15). It was also demonstrated that the cell-permeable diacylglycerol analogue diecanoyl glycerol could mimic the effect of glyceraldehyde on gating of single L-type Ca\textsuperscript{2+} channels in RINm5F cells (52). This led to the suggestion that activation of PKC is part of the stimulus-secretion coupling sequence induced by glyceraldehyde in these cells (53).

Existing data on Ca\textsuperscript{2+} channel activity and PKC activation in the normal pancreatic β-cell are not that extensive, but suggest that activation of PKC with TPA reduces the elevated levels of [Ca\textsuperscript{2+}l] caused by 20 mM glucose and 25 mM K\textsuperscript{+}, the latter effect being dependent on the extracellular glucose concentration (8). It has also been shown that glucose reduces the voltage-threshold for the activation of L-type Ca\textsuperscript{2+} channels (54). Such an effect may be coupled to PKC activation (51–53), since stimulation with glucose increases the synthesis of DAG in β-cells (20). The data showing that direct activation of PKC affects the voltage-gated L-type Ca\textsuperscript{2+} channel in insulin-secreting cells (51–53) are in line with the results obtained in the present study, clearly demonstrating that down-regulation of enzyme activity resulted in a reduction in whole cell Ca\textsuperscript{2+} currents. This reduction resulted in lower peak [Ca\textsuperscript{2+}l] levels subsequent to stimulation with the cell with high concentrations of K\textsuperscript{+}. Although previous observations may suggest that a decrease in PKC activity should increase the voltage threshold for channel activation (51–54), no such effect was observed in the present study. With regard to a possible role of PKC in the regulation of the β-cell stimulus-secretion coupling under physiological conditions and in accordance with the direct effect of the enzyme on the voltage-gated L-type Ca\textsuperscript{2+} channels, it is noteworthy that the [Ca\textsuperscript{2+}l] increase and thereby the activation of the insulin secretory machinery subsequent to glucose stimulation were delayed in normal pancreatic β-cells subjected to PKC down-regulation. Interestingly, the effect of PKC deprivation on insulin release was less accentuated than that on [Ca\textsuperscript{2+}l]. The explanation for this may be of entirely technical nature, i.e. reflect the fact that the measurements of insulin release do not represent a very sensitive method in comparison with those of [Ca\textsuperscript{2+}l], or be due to a true biological difference. If the latter is the case, it may suggest that either the effect of PKC on insulin release is indeed less pronounced than that on the voltage-gated L-type Ca\textsuperscript{2+} channels or that the PKC effect directly on exocytosis is accounted for mainly by the approximately 20% enzyme activity that cannot be down-regulated under the present experimental conditions. Moreover, the results may indicate that the rate and extent of secretion are not a simple function of the [Ca\textsuperscript{2+}l], at any given time.

Our results suggest that the voltage-gated L-type Ca\textsuperscript{2+} channels and thereby the stimulus-secretion coupling in normal

---

**Fig. 3. Immunoblotting of PKC isoenzymes.** The three markings at the far left indicate mobility of (from top to bottom) 97-, 69-, and 46-kDa proteins. Interest should be focussed on the band visible between the 97- and 69-kDa markings. (s, cytosol; p, membrane fraction; t, treated with TPA 200 nM 48 h.) A, blot using antibodies directed versus PKC α. B, blot using antibodies directed versus PKC β.

trol and TPA-treated cells corresponded to 0.32 ± 0.12 min (p < 0.05, n = 6, Student's paired t test).

**DISCUSSION**

Insulin release from the pancreatic β-cell is an example of a complex process regulated by a sophisticated interplay between nutrients and various receptor activating agonists (42). Examples of the latter are those agonists operating through the PLC system, generating InsP\textsubscript{3} and DAG. Thus carbamylcholine (8, 43) and the peptide hormones cholecystokinin (44) and vasopressin (45) cause rapid InsP\textsubscript{3} generation in islets or insulin-secreting tumor cells. The concomitant production of DAG activates PKC (7). Whereas InsP\textsubscript{3} mobilizes intracellularly bound Ca\textsuperscript{2+}, a mechanism which may be involved in promoting oscillations in electrical activity and [Ca\textsuperscript{2+}l] (51), PKC is involved as a modulator of multiple steps regulating the β-cell stimulus-secretion coupling. Most studies evaluating the role of PKC in the insulin secretory process have used the phorbol ester TPA as an activator of the enzyme. However, activation of PKC with TPA produces a much stronger and more long-lived effect than that produced by DAG under physiological conditions (47). Another approach when evaluating effects of PKC on the β-cell stimulus-secretion coupling is to deplete the cell of the active enzyme under different experimental conditions (8, 17, 22, 39).

When acutely activating PKC with TPA in normal pancreatic β-cells, the present study shows an inhibition of agonist-induced InsP\textsubscript{3} formation. A similar inhibition has previously been obtained in insulin-secreting tumor cell lines as well as other cell types (39–41, 48, 49). The effect of PKC on the PLC system may be accounted for by interference with the receptor-linked G-protein, the PLC itself or other, as yet, undefined steps is this complex receptor-operated pathway (17, 39, 47). When instead down-regulating PKC, by long-term TPA treatment, there was an increased production of InsP\textsubscript{3} subsequent to stimulation with carbamylcholine. This effect was paralleled by a larger agonist-induced [Ca\textsuperscript{2+}l] response, compared to control conditions. These data suggest that PKC under physiological conditions, in addition to serving as a negative feed-back modulator of receptor responses mediated by PLC, is tonically active in inhibiting the PLC system in the pancreatic β-cell. There are no reasons to believe that such a tonic activation of PKC is a specific feature of β-cells obtained from pancreatic islets enriched in insulin-secreting cells; pancreatic islets from ob/ob mice containing more than 90% β-cells (26).

Existing data on Ca\textsuperscript{2+} channel activity and PKC activation in the normal pancreatic β-cell are not that extensive, but suggest that activation of PKC with TPA reduces the elevated levels of [Ca\textsuperscript{2+}l] caused by 20 mM glucose and 25 mM K\textsuperscript{+}, the latter effect being dependent on the extracellular glucose concentration (8). It has also been shown that glucose reduces the voltage-threshold for the activation of L-type Ca\textsuperscript{2+} channels (54). Such an effect may be coupled to PKC activation (51–53), since stimulation with glucose increases the synthesis of DAG in β-cells (20). The data showing that direct activation of PKC affects the voltage-gated L-type Ca\textsuperscript{2+} channel in insulin-secreting cells (51–53) are in line with the results obtained in the present study, clearly demonstrating that down-regulation of enzyme activity resulted in a reduction in whole cell Ca\textsuperscript{2+} currents. This reduction resulted in lower peak [Ca\textsuperscript{2+}l] levels subsequent to stimulation with the cell with high concentrations of K\textsuperscript{+}. Although previous observations may suggest that a decrease in PKC activity should increase the voltage threshold for channel activation (51–54), no such effect was observed in the present study. With regard to a possible role of PKC in the regulation of the β-cell stimulus-secretion coupling under physiological conditions and in accordance with the direct effect of the enzyme on the voltage-gated L-type Ca\textsuperscript{2+} channels, it is noteworthy that the [Ca\textsuperscript{2+}l] increase and thereby the activation of the insulin secretory machinery subsequent to glucose stimulation were delayed in normal pancreatic β-cells subjected to PKC down-regulation. Interestingly, the effect of PKC deprivation on insulin release was less accentuated than that on [Ca\textsuperscript{2+}l]. The explanation for this may be of entirely technical nature, i.e. reflect the fact that the measurements of insulin release do not represent a very sensitive method in comparison with those of [Ca\textsuperscript{2+}l], or be due to a true biological difference. If the latter is the case, it may suggest that either the effect of PKC on insulin release is indeed less pronounced than that on the voltage-gated L-type Ca\textsuperscript{2+} channels or that the PKC effect directly on exocytosis is accounted for mainly by the approximately 20% enzyme activity that cannot be down-regulated under the present experimental conditions. Moreover, the results may indicate that the rate and extent of secretion are not a simple function of the [Ca\textsuperscript{2+}l], at any given time.

Our results suggest that the voltage-gated L-type Ca\textsuperscript{2+} channels and thereby the stimulus-secretion coupling in normal
pancreatic ß-cells are under dynamic influence of PKC-induced phosphorylation. The fact that the voltage-gated Ca\(^{2+}\) currents can be increased by PKC activation (51-53) and decreased by PKC down-regulation (the present study), suggests that this enzyme has a modulatory function even under basal conditions. We conclude that PKC probably has multiple roles and thereby interacts at various steps in the ß-cell signal transduction pathway. We suggest that one important role for the kinase is to maintain the functional state of the L-type voltage-activated Ca\(^{2+}\) channels in the pancreatic ß-cell. Moreover, if the open state probability of these channels is altered from moment to moment by the degree of PKC activity, receptor activation of the PLC system will serve as a potent modulator of glucose-induced insulin release. To what extent both the \(\alpha\) and the \(\beta_1\) isozymes of PKC are responsible for the effects on the Ca\(^{2+}\) channel is at the moment not clear, since both of them were down-regulated by prolonged TPA treatment.

Acknowledgment—We are grateful to Dr. Peter Parker for providing the anti-PKC antiserum.

REFERENCES


FIG. 4. Dynamics of insulin release from pancreatic ß-cells. A and B, control cells are shown in the upper graphs and cells treated with 200 nM TPA for 24-48 h in the lower graphs. Glucose was introduced as indicated by the arrows. In A insulin release is expressed relative to the mean insulin release during the first five fractions (100%). Mean values ± S.E. for six experiments. In B one of these experiments is shown, with the curve resulting from the fitted polynomial function included in the graph. The vertical dashed lines indicate the peaks of insulin release, obtained from the polynomial functions. The shaded area represents the actual difference in time between control and TPA-treated cells. B also gives the absolute amounts of insulin released in this experiment (indicated to the right). C, upper panel shows the time difference in peak insulin release between control and TPA-treated cells (mean Δ time ± S.E.; n = 6). Lower panel shows the actual times from glucose stimulation to peak insulin release for the two groups of cells (mean ± S.E.; n = 6).
Protein Kinase C and the Insulin Secretory Process


