Insulinotropic Effect of the Tumor Promoter 12-O-Tetradecanoylphorbol-13-Acetate in Rat Pancreatic Islets

W. J. Malaisse, A. Sener, A. Herchuelz, A. R. Carpinelli, P. Poloczek, J. Winand, and M. Castagna

Laboratories of Experimental Medicine [W. J. M., A. S., A. R. C.], Pharmacology [A. H.], and Biochemistry [P. P., J. W.], Brussels University, Brussels, Belgium; and Institute for Scientific Research on Cancer, Villejuif, France [M. C.]

ABSTRACT

In isolated rat pancreatic islets, the tumor-promoting agent 12-O-tetradecanoylphorbol-13-acetate (TPA), when used in the 2.10^{-9} to 2.10^{-7} M range, was found to stimulate insulin release both in the absence and presence of glucose. The non-tumor-promoting agent 4-methylphorbol-12,13-didecanoate failed to stimulate insulin release. The insulinotropic capacity of TPA was enhanced by glucose in a dose-related fashion. In the absence of glucose, the TPA-stimulated release of insulin was a slowly induced and not rapidly reversible phenomenon. It was inhibited by antimycin A, by epinephrine, at low temperatures, and in the absence of extracellular Ca^{2+} or the presence of cytochalasin B, was unaffected by the organic calcium antagonist D600 and indomethacin, and was potentiated by theophylline. No obvious effect of TPA upon 86Rb or 32P efflux and 45Ca net uptake could be detected in the isolated islets. However, TPA caused a progressive increase in both 45Ca fractional outflow rate and cyclic adenosine 3':5'-monophosphate content in the islets. It is proposed that the insulinotropic action of TPA may be due, in part at least, to interference with the transport of calcium by native ionophores.

INTRODUCTION

The tumor promoter TPA exerts early cellular effects on many different cell types (29). TPA was recently reported to stimulate insulin release from isolated rat islets (33). The present study represents an attempt to elucidate the mode of action of TPA in the pancreatic B-cell.

MATERIALS AND METHODS

Islets of Langerhans were isolated from the pancreas of fed female albino rats (13). The methods used to measure insulin release (17), glucose oxidation (23), and 45Ca net uptake (25) in incubated islets and 86Rb (3), 45Ca (12), and 32P (7) FOR from perfused islets were all previously described. For the measurement of cyclic AMP, groups of 40 islets each were incubated for 3, 15, or 60 min in 0.1 ml of incubation medium. The tubes containing the islets were placed in liquid N2 and the islets were disrupted by mechanical vibration at 400 to 800 cycles/sec after addition of 80 μl of trichloroacetic acid (22.5% (w/v)]. After centrifugation, the supernatant solution was mixed with 10 μl HCl (1.0 N) and extracted 5 times with H2O-saturated ethyl ether (1 ml). After evaporation of remaining ether, the homogenate was transferred to small tubes (6 x 50 mm), frozen, and lyophilized. The lyophilized material was resuspended in 50 μl H2O and assayed in duplicate (20 μl each) by the method of Gilman (10).

TPA (P. Borchert, Eden Prairie, Minn.) and 4-methylphorbol-12,13-didecanoate (gift of Dr. E. Hecker, Heidelberg, West Germany) were added to the incubation or perfusion media from stock solutions (0.15 to 1.62 mM) prepared in dimethyl sulfoxide. The same final concentration of the solvent (<1.25 μl/ml) was present in control media containing no drug. At these low concentrations, dimethyl sulfoxide fails to affect metabolic and secretory variables in the islets (14). All results are expressed as the mean ± SE together with the number of individual determinations (n).

RESULTS

Effect of TPA upon Insulin Release. TPA at a concentration of 1.6 nM failed to stimulate insulin release, whether in the absence or presence of glucose (Table 1). At higher concentrations (16 and 162 nM), TPA caused a dose-related stimulation of insulin release, both in the absence and presence of glucose. The sensitivity to increasing concentrations of TPA appeared dependent on the concentration of glucose. Thus, the increase in insulin output attributable to 16 nM TPA, relative to that evoked by 162 nM TPA, increased from 33.4 ± 9.0 to 53.0 ± 8.9 and 79.9 ± 12.9% as the glucose concentration was raised from zero to 5.6—8.3, and 16.7 mM, respectively. The magnitude of the increment in insulin output attributable to TPA was also modulated by the glucose concentration of the incubation medium (Chart 1, left). Thus, in the presence of 16 and 162 nM TPA, the drug-induced increment in insulin output, relative to that seen at the same concentration of the drug in the presence of 16.7 mM glucose, increased from 29.9 ± 3.8 to 50.9 ± 4.7 and 100.0 ± 10.1% as the glucose concentration was raised from zero to 5.6—8.3, and 16.7 mM, respectively.

When islets were incubated for 30, 60, and 90 min in the absence of glucose, the time course for TPA-induced insulin release was suggestive of a progressive and sustained stimulant action of the drug, with a mean increment in insulin output of 483 ± 50 nanounits/islet/min (Chart 1, right). This was confirmed in perfused islets, with TPA causing a slowly progressing rise in insulin output. By paired comparison, the TPA-induced increment in insulin output above basal value (31 to 44 min) (Chart 2, bottom) was significant (p < 0.05) after an 8-min exposure to the drug (Δ = 54.5 ± 6.3 nanounits/islet/ min). When TPA was removed from the perfusate, the output
Table 1

Effect of TPA upon insulin output at different glucose concentrations

<table>
<thead>
<tr>
<th>Glucose Concentration</th>
<th>Control</th>
<th>TPA (1.6 nM)</th>
<th>TPA (16.2 nM)</th>
<th>TPA (161.6 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No glucose</td>
<td>18.2 ± 2.7 (10)</td>
<td>6.9 ± 2.3 (10)</td>
<td>30.3 ± 5.3 (10)</td>
<td>64.7 ± 7.6 (10)</td>
</tr>
<tr>
<td>6.6 mM glucose</td>
<td>5.6 ± 2.3 (9)</td>
<td>27.6 ± 9.6 (9)</td>
<td>60.6 ± 4.4 (10)</td>
<td>117.9 ± 17.7 (10)</td>
</tr>
<tr>
<td>8.3 mM glucose</td>
<td>5.6 ± 2.3 (9)</td>
<td>27.6 ± 9.6 (9)</td>
<td>60.6 ± 4.4 (10)</td>
<td>117.9 ± 17.7 (10)</td>
</tr>
<tr>
<td>16.7 mM glucose</td>
<td>5.6 ± 2.3 (9)</td>
<td>27.6 ± 9.6 (9)</td>
<td>60.6 ± 4.4 (10)</td>
<td>117.9 ± 17.7 (10)</td>
</tr>
</tbody>
</table>

Secretory rate significantly higher than control values (p < 0.001).

Mean ± S.D. Numbers in parentheses, number of individual determinations.

Insulin output (pmoles/islet/90 min)

Effects of TPA upon Glucose Oxidation, Ionic Variables, and Cyclic AMP Content. TPA slightly but not significantly inhibited glucose oxidation in the islets (Table 3). The drug failed to exert any obvious effect upon 45Ca FOR and 32P FOR from perfused islets (Chart 3) and upon 45Ca net uptake by incubated islets (Table 3).

TPA, however, caused a slowly progressing rise in 45Ca FOR from prelabeled islets perfused in the absence or presence of extracellular Ca2+ (Chart 2). The latter effect of TPA was reversed, in part at least, when TPA was removed from the perfusate. For instance, in the absence of extracellular Ca2+, the rate of change in 45Ca FOR, as estimated from the regression coefficient (± its sample S.D.) of FOR versus time, averaged −200 ± 11, +99 ± 7, and −18 ± 1/million/min before, during, and after exposure to TPA, respectively. Likewise, in the presence of extracellular Ca2+, the rate of change in 45Ca FOR averaged −114 ± 7, +47 ± 3, and −48 ± 5/million/min before, during, and after TPA administration, respectively. Relative to the mean control FOR found just prior to TPA administration, the TPA-induced increase in calcium outflow was almost identical in the absence or presence of Ca2+, corresponding, respectively, to a 2.93 ± 0.19 and 3.01 ± 0.19% increase per min in 45Ca FOR (p < 0.001 in both cases).

During incubation in a glucose-free medium, TPA (0.2 μM) caused a slowly induced rise in the cyclic AMP content of the islets and their surrounding medium. Relative to the paired control value found at the same time in the absence of TPA, the cyclic AMP content of the TPA-stimulated islets averaged...
**Insulinotropic Effect of TPA**

**Table 2**
Effect of TPA and other agents upon insulin release by islets incubated in the absence of glucose

<table>
<thead>
<tr>
<th>Line</th>
<th>TPA (μM)</th>
<th>Me-PDDb (μM)</th>
<th>Other agents</th>
<th>Insulin output (μunits/islet/90 min)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td>15.3 ± 2.4c (29)c</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td></td>
<td>Antimycin A (10 μM)</td>
<td>16.2 ± 2.9 (10)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td></td>
<td>4-</td>
<td>71.2 ± 3.4 (59)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td></td>
<td>Epinephrine (5.5 μM)</td>
<td>15.6 ± 2.3 (9)</td>
<td>&lt;0.001 vs. 1</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td></td>
<td>No Ca2⁺ + EGTA (0.5 mM) + theophylline (1.4 mM)</td>
<td>11.5 ± 1.8 (9)</td>
<td>&lt;0.001 vs. 3</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td></td>
<td>No Ca2⁺ + EGTA (0.5 mM)</td>
<td>28.2 ± 2.8 (18)</td>
<td>&lt;0.001 vs. 3</td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
<td></td>
<td>D600 (80 μM)</td>
<td>47.3 ± 3.0 (9)</td>
<td>&lt;0.001 vs. 7</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
<td></td>
<td>Indomethacin (10 μM)</td>
<td>169.1 ± 6.0 (10)</td>
<td>&lt;0.001 vs. 3</td>
</tr>
<tr>
<td>9</td>
<td>0.2</td>
<td></td>
<td>Theophylline (1.4 mM)</td>
<td>80.2 ± 2.8 (9)</td>
<td>NS vs. 3</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td></td>
<td>Cytochalasin B (21 μM)</td>
<td>76.4 ± 8.7 (10)</td>
<td>NS vs. 3</td>
</tr>
<tr>
<td>11</td>
<td>0.2</td>
<td></td>
<td></td>
<td>51.2 ± 4.9 (9)</td>
<td>&lt;0.05 vs. 3</td>
</tr>
<tr>
<td>12</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Me-PDD, 4-methylphorbol-12,13-didecanoate; NS, not significant; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N’-tetracetic acid.
b Mean ± S.E.
c Numbers in parentheses, number of individual determinations.

**Table 3**
Effect of TPA upon glucose oxidation and Ca2⁺ net uptake, and cyclic AMP content in isolated islets

<table>
<thead>
<tr>
<th>TPA</th>
<th>Ca net uptake (pmol/islet/90 min)</th>
<th>[U-14C]Glucose oxidation (pmol/islet/120 min) with 16.7 mm glucose</th>
<th>Cyclic AMP (pmol/islet/60 min) with no glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>1.14 ± 0.07 (12)</td>
<td>5.48 ± 0.45 (12)</td>
<td>55.9 ± 3.7 (12)</td>
</tr>
<tr>
<td>0.2 μM</td>
<td>1.09 ± 0.08 (12)</td>
<td>5.20 ± 0.22 (12)</td>
<td>49.6 ± 4.0 (12)</td>
</tr>
</tbody>
</table>

a Mean ± S.E.
b Numbers in parentheses, number of individual determinations.

drug-induced increment in insulin output were augmented in a dose-related fashion as a function of the ambient glucose concentration. In this respect, TPA acts as both a glucose-simulating and glucose-potentiating agent, as defined elsewhere (15). Indeed, the drug is able to stimulate insulin release in the absence of glucose (glucose-simulating effect), but exerts its most marked enhancing action at high glucose concentrations (glucose-potentiating effect). Such dual behavior is quite unusual, being shared to some extent only by hypoglycemic sulfonylureas (22).

Because the major aim of the present study was to elucidate the mode of action of TPA in the islet cells, we have concentrated our attention on the process of TPA-induced release as observed in the absence of glucose. It is obvious that TPA does not augment glucose-induced insulin release by facilitating glucose metabolism in the islets. On the contrary, TPA slightly decreased glucose oxidation by the islets. It is also unlikely that TPA acts primarily by facilitating the metabolism of endogenous nutrients, in which case a decrease in [86Rb] FOR and possibly a phosphate flush would be expected to occur (4, 8). TPA failed to have any effect on [86Rb] and [32P] FOR from the islets.

The release of insulin evoked by TPA in the absence of glucose represented a slowly initiated, sustained, and not rapidly reversible phenomenon. It was inhibited by antimycin A and at low temperature, suggesting that it corresponded to an active, energy-dependent process of secretion.

It could be proposed that TPA stimulates insulin release through a primary effect on the synthesis or catabolism of cyclic AMP. However, agents which exert such a primary effect (e.g., glucagon or theophylline) do not cause a sustained stimulation of insulin release in the absence of another secretagogue, such as glucose (2). A role for cyclic guanosine 3':

**DISCUSSION**

The present results confirm that TPA is a potent insulin secretagogue (33). At variance with a previous report (33), TPA was found to stimulate insulin release both in the absence and presence of glucose. Nevertheless, both the sensitivity to increasing concentrations of TPA and the magnitude of the TPA (0.16 μM) administered from the times shown (---) upon 86Rb top, n = 3 and 32P bottom, n = 2 FOR (mean ± S.E.) islets perfused with glucose-free media containing 1.0 mm Ca²⁺.

99.3 ± 2.9% (n = 6; not significant), 113.9 ± 4.6% (n = 6; p < 0.05), and 171.6 ± 24.3% (n = 12; p < 0.05) after 3-, 15-, and 60-min incubations, respectively.

**OCTOBER 1980**

3829
5'-monophosphate in TPA-induced insulin release, as proposed by Virji et al. (33), cannot be ruled out, although no evidence has yet been obtained to indicate that cyclic guanosine 3':5'-monophosphate exerts any insulinotropic action (9). Incidentally, the fact that TPA increased the cyclic AMP content of the islets and their surrounding medium could be secondary to the effects of the drug upon calcium handling by the islet cells (see below).

The failure of indomethacin to affect TPA-stimulated insulin release does not suggest a key role for endogenous prostaglandins in such a process.

An alternative hypothesis would be that TPA exerts a primary effect upon ionic fluxes in the islet cells. TPA failed to affect 86Rb+ fractional outflow rate from the islets. This is in sharp contrast with the effect of several insulinotropic nutrients (e.g., glucose, leucine, α-ketoisocaproate), which decrease K+ conductance and, by doing so, cause a depolarization of the plasma membrane and the subsequent gating of voltage-dependent Ca2+ channels.

It is currently thought that insulin release is triggered by the accumulation of Ca2+ in the cytosol of the B-cell close to the cell boundary (27), whatever secretagogue is used to stimulate secretion (19). TPA could provoke Ca2+ accumulation by affecting the transport of this cation across membrane systems in the islet cells. This view is compatible with the finding that, in an artificial system for the study of ionophoresis (24), TPA enhances the translocation of calcium into a hydrophobic domain, as mediated by the ionophore A23187 or native ionophoretic material extracted from the islets.

It could be argued that an effect of TPA in facilitating calcium transport in the islet cells is not compatible with our data on 45Ca net uptake. It should be noted, however, that these data refer to measurements performed under conditions close to isotopic equilibrium (21) and, therefore, encompass much larger calcium compartments than the sole cytosolic pool of ionized Ca2+. In other words, our data on 45Ca net uptake refer to apparent mm concentration of calcium in the islet cells, whereas a minute increase in cytosolic Ca2+ (in the μM range) may be sufficient to cause insulin release. A TPA-induced facilitation of ionophore-mediated calcium transport could account for a progressive and localized increase in cytosolic Ca2+ concentration, leading to a delayed stimulation of insulin release. This model would differ vastly from the normal process of nutrient-induced insulin secretion, in which a rapid gating of calcium channels is accompanied by an equally rapid secretory response and by an obvious increase in 45Ca net uptake.

Several observations in the present report support the view that TPA stimulated insulin release by facilitating the translocation of calcium from the extracellular fluid and/or intracellular organelles into the cytosolic compartment. (a) The secretory response to TPA was abolished by epinephrine, which is claimed to cause the uptake and sequestration of calcium by the vacuolar system of the B-cell (1). (b) TPA-induced insulin release was inhibited in the absence of extracellular Ca2+. (c) Whether in the absence or the presence of extracellular Ca2+, theophylline, which apparently mobilizes calcium from the vacuolar system (2), potentiated the secretory response to TPA. In the present system, theophylline alone does not stimulate insulin release from glucose-deprived islets (2).

The failure of D600 to significantly affect TPA-stimulated insulin release, the modest inhibitory effect of cytochalasin B upon such a process, and the effect of TPA to increase the cyclic AMP content of the islets are all also compatible with the present hypothesis. (a) The process of calcium inflow in the islet cells may be protected by TPA against the inhibitory action of D600, since the tumor promoter and the organic calcium antagonist apparently exert opposite effects upon ionophore-mediated calcium translocation in an artificial system. Moreover, organic calcium antagonists such as D600 do not affect the capacity of drugs to mobilize Ca2+ from intracellular storage sites (20, 30). (b) Cytochalasin B, which invariably enhances insulin release evoked by nutrient secretagogues (14), tends to inhibit secretion whenever the secretory response is triggered by a primary change in divalent cation transport (28). (c) The TPA-induced accumulation of cyclic AMP may well be secondary to the increase in cytosolic Ca2+ concentration, since in the islets adenylate cyclase activity is stimulated by endogenous calmodulin in a Ca2+-dependent fashion (32).

The postulated primary effect of TPA in facilitating the carrier-mediated transport of calcium across membranes in the B-cell is supported by the finding that TPA increased 45Ca FOR in perfused islets. The fact that such an increase occurred both in the absence and presence of extracellular Ca2+ suggests that it corresponds to a true facilitation of calcium efflux rather than stimulation of a process of calcium-calcium exchange (11). The outflow of calcium from the islet cells is currently ascribed to a carrier-mediated process of sodium-calcium countertransport (12). Ionophores such as A23187 or X537A are able to mimic the latter process (16, 18). TPA might affect the environment of native ionophores (31) or act in cooperation with them to facilitate such a process. A cooperative behavior between distinct ionophores was recently identified (5, 6).

In conclusion, TPA may stimulate insulin release, in part at least, by interfering with calcium transport, as mediated by native ionophores in the B-cell. It is tempting to consider that such a modality of action may also be operative in other biological Systems affected by this tumor-promoting agent.

ACKNOWLEDGMENTS

The authors are grateful to C. Rémond, A. Tinant, J. Schoonheydt, and M. Urbain for technical assistance; and to C. Demesmaeker and S. Procureur for secretarial help.

REFERENCES

Insulinotropic Effect of the Tumor Promoter 12-O-Tetradecanoylphorbol-13-Acetate in Rat Pancreatic Islets

W. J. Malaisse, A. Sener, A. Herchuelz, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/10/3827

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/40/10/3827.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.