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TUMOR PROMOTER 12-O-TETRADECANOYLPHORBOL-13-ACETATE-INDUCED INSULIN SECRETION: INHIBITION BY PHOSPHOLIPASE A_2- AND LIPOXYGENASE-INHIBITORS

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SUMMARY: In isolated pancreatic islets of rats, an insulin secretion was induced by a tumor promoter 12-O-tetradecanoylphorbol-13-acetate in a low glucose medium. The insulin secretion was inhibited by p-bromophenacyl bromide, mepacrine, nordihydroguaiaretic acid and 1-phenyl-3-pyrazolidinone but not by indomethacin. When the insulin secretion was suppressed by p-bromophenacyl bromide, the secretion was partially but not significantly restored by lysophosphatidyl choline and was fully restored by the simultaneous addition of arachidonic acid and lysophosphatidyl choline. These results suggest that activation of phospholipase A_2 and a lipoxygenase product(s) play important roles in the 12-O-tetradecanoylphorbol-13-acetate-induced insulin secretion.

A tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA)[1], exerts various biological and biochemical effects on many different cell types (1,2). Insulinotropic effect of TPA has been recently demonstrated, using isolated pancreatic islets (3-5). Our recent findings suggest that an activation of phospholipase A_2 and a lipoxygenase product(s) are involved in the secretory mechanism of glucose-induced insulin secretion from rat pancreatic islets (6). It has been well-established that TPA enhances phospholipid deacylation by activating phospholipase A_2 (7-10). Thus, we attempted to test whether

Footnotes: [1] The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; NDGA, nordihydroguaiaretic acid; BPB, p-bromophenacyl bromide; Phenidone, 1-phenyl-3-pyrazolidinone.

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phospholipase A_2 activation and resultant arachidonate metabolites are involved in the TPA-induced insulin secretion using isolated rat pancreatic islets.

MATERIALS AND METHODS

Materials Collagenase (Type V), crystallized and lyophilized bovine serum albumin, nordihydroguaiaretic acid, indomethacin, mepacrine (quinacrine) dihydrochloride, arachidonic acid sodium salt (from porcine liver), L-α-lysophosphatidyl choline (from egg yolk) and 12-O-tetradecanoylphorbol-13-acetate were purchased from Sigma Chemical Co., St. Louis, MO. p-Bromophenacyl bromide and 1-phenyl-3-pyrazolidinone were obtained from Wako Pure Chemical Ind. Ltd., Osaka, Japan, and Nakarai Chemicals Ltd., Kyoto, Japan, respectively.

Experiments with isolated pancreatic islets Male Sprague-Dawley rats weighing 300 to 350 g were used. Pancreatic islets were isolated by the method of Lacy and Kostianovsky (11) with minor modifications (12). Isolated pancreatic islets were transferred into a teflon meshed basket (5 islets/basket) in a flask containing 0.5 ml of 95% O_2 and 5% CO_2 saturated Krebs-Ringer bicarbonate solution (pH 7.4) of the following composition (mM): 120 NaCl, 4.8 KCl, 25.5 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.7 $CaCl_2$ and 3.3 glucose supplemented with 0.2% crystallized and lyophilized bovine serum albumin. Islets were preincubated by shaking at 90 strokes/min for 40 min at 37° C. After preincubation, the medium was changed to a new solution containing the indicated concentrations of glucose and various drugs. Incubation was continued for another 60 min to measure insulin release. To determine the effects of various inhibitors, islets were preincubated for 30 min with mepacrine or indomethacin, for 15 min with NDGA or phenidone and for 7 min with BPB. The above drugs, except BPB, were added to the final incubation medium as well as to the preincubation medium. Released insulin in the medium was assayed by the commercially available insulin kit for radioimmunoassay (Dainabot RI Institute Co., Tokyo, Japan).

RESULTS AND DISCUSSION

Virji et al., (3) first reported the enhancing effect of TPA on the glucose-induced insulin release from isolated rat pancreatic islets. They observed the failure of TPA to initiate the insulin release in the absence or subthreshold concentration of glucose. Malaisse et al., (4), however, claimed that even in the absence of glucose, TPA can induce insulin secretion. The present data also demonstrate that TPA induces the insulin secretion in a concentration dependent-manner in a medium whose glucose concentration is subthreshold level (3.3 mM) (Table 1).

Table 1. Insulinotropic effect of TPA in isolated pancreatic islets[a)]

TPA (μM)	Insulin[b)] μU/islet/60 min
0	43 ± 9
0.1	87 ± 17
0.3	121 ± 22
1	207 ± 30
3	281 ± 56

a) Glucose concentration of the medium was 3.3 mM.
b) Mean ± S.E. (n=4-5)

Recently we demonstrated the possible involvement of phospholipase A_2 activation and a lipoxygenase product(s) in the mechanism of glucose-induced insulin secretion (6). TPA has been shown to enhance arachidonic acid release by stimulating phospholipase A_2 in various cell types (7-10). As shown in Table 2, phospholipase A_2 inhibitors, such as mepacrine (0.1 mM) (13,14) and BPB (0.1 mM) (15,16) inhibited the TPA-induced insulin secretion.

Table 2. Effects of several antagonists on TPA-induced insulin secretion in isolated pancreatic islets[a)]

	Insulin[b)] μU/islet/60 min
None	43 ± 9**
TPA 1 μM	225 ± 31
TPA 1 μM + Mepacrine 0.1 mM	60 ± 14**
TPA 1 μM + BPB 0.1 mM	56 ± 12**
TPA 1 μM + Indomethacin 10 μM	231 ± 18
TPA 1 μM + NDGA 0.1 mM	47 ± 14**
TPA 1 μM + Phenidone 0.1 mM	50 ± 21**

a) Glucose concentration of the medium was 3.3 mM.
b) Mean ± S.E. (n=5)
** Significantlly different from the data with 1 μM TPA ($p<0.01$).

The results indicate the involvement of phospholipase A_2 activation in the mechanism of TPA-induced insulin secretion.

Since isolated pancreatic islet is capable of metabolizing arachidonic acid to lipoxygenase pathway as well as to cyclooxygenase pathway (17), it is reasonable to assume that the phospholipase A_2-activation by TPA causes the formation of both cyclooxygenase- and lipoxygenase-products. As shown in Table 2, TPA-induced insulin secretion was not affected by 10 μM indomethacin, a cyclooxygenase inhibitor. But 0.1 mM NDGA and 0.1 mM phenidone, which inhibit lipoxygenase or both cyclooxygenase and lipoxygenase (18-21), did inhibit the TPA-induced insulin secretion (Table 2). These results indicate that a lipoxygenase product(s) rather than a cyclooxygenase product(s) plays an important role in the TPA-induced insulin secretion.

When the TPA-induced insulin secretion was inhibited by BPB, the insulin secretion could neither be restored by the addition of arachidonic acid (0.2 mM) nor by the combination of arachidonic acid (0.2 mM) with indomethacin (5 μM) (Fig. 1). The insulin secretion was partially but not significantly restored by the addition of another phospholipase A_2 product, lysophosphatidyl choline (60 μg/ml) (Fig. 1). Under the same experimental conditions, the simultaneous addition of arachidonic acid (0.2 mM), indomethacin (5 μM) and lysophosphatidyl choline (60 μg/ml) caused a full restoration of the insulin secretion (Fig. 1). Since the restoration was observed either in the presence (Fig. 1) or absence (data not shown) of indomethacin, the restoring effect of arachidonic acid can be attributable to its lipoxygenase product(s) or non-metabolized form of arachidonic acid. According to the data of Croce et al.(22), using several types of cells, lysophosphatidyl choline at a concentration of 100 μg/ml has no effect on the viability of cells. In our experimental conditions, 60 μg/ml of lysophosphatidyl

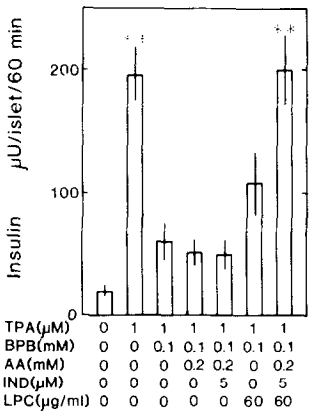

Fig. 1. Effects of arachidonic acid and lysophosphatidyl choline on the inhibition of TPA-induced insulin secretion by BPB. Isolated pancreatic islets were preincubated with or without 0.1 mM BPB for 7 min in a low glucose (3.3 mM) medium. After preincubation, each medium was changed to a new medium (3.3 mM glucose) which contained indicated concentrations of TPA, arachidonic acid (AA), indomethacin (IND) and lysophosphatidyl choline (LPC) but no BPB. Incubation was continued for another 60 min to measure insulin release. Each value represents the mean ± S.E. (n=5). ** $p<0.01$ vs TPA + BPB.

choline alone failed to induce insulin secretion from the islets (data not shown). Thus, it is unlikely that cell lytic properties of lysophosphatidyl choline contribute to the above effect. Therefore, our results further support the contention that activation of phospholipase A_2 is involved in the mechanism of TPA-induced insulin secretion, and also suggest that lysophosphatidyl choline or other lysophospholipids formed by the activation of phospholipase A_2, may possibly be involved in the secretory mechanism. It has been proposed that lysophospholipids are involved in the exocytotic secretion by inducing the fusion of the membrane of the secretory organelle with the plasma membrane (23-25). Since insulin is secreted by exocytotic process, it is possible that lysophospholipids, such as lysophosphatidyl choline, are involved in the secretory mechanism.

Malaisse and his colleagues (4,5) claimed that TPA stimulates insulin release by facilitating the translocation of calcium from

the extracellular fluid and/or intracellular organelle into the cytosolic compartment. Our present data do not necessarily deny that implication, since at least in granulocytes, lipoxygenase products play a significant role in the membrane calcium flux (26-28). Recently it has been reported that lipoxygenase products are involved in the exocytotic degranulation of specific granules from neutrophils (29-30) and the secretory mechanism of histamine in mast cells (31) and basophils (32,33). Thus it may be possible that lipoxygenase products contribute in a similar way to the secretory process of insulin in pancreatic islets.

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