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STIMULATION OF HEPATIC GLYCOGENOLYSIS BY 12-O-TETRADECANOYL-PHORBOL-13-ACETATE (TPA) VIA CYCLOOXYGENASE PRODUCTS

J. Adolfo García-Sáinz* and S. M. Teresa Hernández-Sotomayor

Departamento de Bioenergética, Instituto de Fisiología Celular
and Departamento de Bioquímica, Facultad de Medicina;
Universidad Nacional Autónoma de México,
Apartado Postal 70-600; 04510 México D. F.

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12-O-Tetradecanoyl-phorbol-13-acetate (TPA) stimulates glycogenolysis in perfused rat liver. The effect of TPA was blocked by indomethacin and bromophenacyl bromide. The effect of TPA on glucose output was transient in spite of the continuous presence of the phorbol ester in the perfusion medium. Addition of platelet activating factor (PAF) after the effect of TPA did not stimulate glycogenolysis. In contrast, vasopressin was able to stimulate glucose output under these conditions. Interestingly, as previously reported, PAF produced also transient stimulation of glycogenolysis; the addition of TPA after the effect of PAF had declined, was also unable to increase glucose output by the liver. It is suggested that both PAF and TPA stimulate hepatic metabolism through the generation of cyclooxygenase products.

Phorbol esters are a series of diterpene tumor promoters which seem to exert their actions through activation of protein kinase C (1,2). This enzyme is proposed to mediate, at least partially, the actions of hormones and neurotransmitters that act through phosphoinositide breakdown and calcium signalling (1-3). Nevertheless, we (4) and others (5,6) have observed that 12-O-tetradecanoyl-phorbol-13-acetate (TPA), the most potent of these tumor promoters, is unable to reproduce in isolated hepatocytes the metabolic actions of hormones that act through calcium.

Interestingly, however, it has been recently reported that TPA stimulates hepatic glycogenolysis in perfused liver (7). Thus, the results obtained with TPA in liver cells as compared to those using perfused liver are apparently contradictory.

*To whom correspondence should be addressed.

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A similar apparent contradiction has been observed regarding the action of platelet-activating-factor (PAF). This lipid, stimulates glycogenolysis in perfused liver (8-12) but is unable to do so in isolated hepatocytes (10, 12). These data suggest that cells besides hepatocytes are involved in the effect of this agent and that such cells may release a mediator that then activates the hepatocytes (12). Furthermore, it has been observed that cyclooxygenase inhibitors block the action of PAF in perfused liver (12).

Taking into account these data, the effect inhibitors of phospholipase A_2 and cyclooxygenase on the action of TPA in perfused rat liver was studied. Our data suggest that the glycogenolytic effect of TPA involves cyclooxygenase products and that TPA and PAF desensitize each other, which suggest that both lipids share a common pathway for activating hepatic glycogenolysis.

MATERIALS AND METHODS

12-O-Tetradecanoyl-phorbol-13-acetate (TPA), 1-alpha, phosphatidylcholine, beta-acetyl-gamma-O-alkyl (PAF), 4-bromophenacyl bromide (BPB), indomethacin (INDO) and arginine-vasopressin were obtained from Sigma Chemical Co. Bovine serum albumin (fraction V) was obtained from Armour. Other reagent were of reagent grade.

Experiments were performed with female Wistar rats (approx. 200 g) fed ad libitum with Purina rat chow. Liver was perfused using a nonrecirculating hemoglobin-free perfusion system (12). The perfusion medium was Krebs-Ringer bicarbonate buffer saturated with O_2/CO_2 (95% / 5%) pH 7.4 maintained at 37°C. The calcium chloride concentration was 1.3 mM. The perfusions were carried out in the morning between 8 and 11 a.m.. The livers were perfused 45 min prior to the addition of the agents to be tested, the last 15 min of this equilibration in the presence of 0.25% albumin. To determine the effects of inhibitors, the liver was perfused 15 min before the addition of TPA with INDO (100 μM), or BPB (100 μM) and were present throughout the experiment. The efficient perfusate was collected at 1 min intervals and glucose was assayed by the glucose oxidase procedure (13). Results are expressed as percentage of basal glucose output corrected for the flow rate per minute and the wet weight of the liver.

RESULTS

Addition of TPA (10 ng/ml) stimulate glycogenolysis in perfused rat liver (Fig. 1) the effect of TPA reached it maximum 5-10 min after addition to the perfusion medium and persisted for 20 min. After this time, in spite of the continuous presence of the drug, glycogenolysis returned to basal values. Furthermore, addition of more TPA did not increased glycogenolysis. This data indicates that the liver desensitizes to TPA. However, the addition

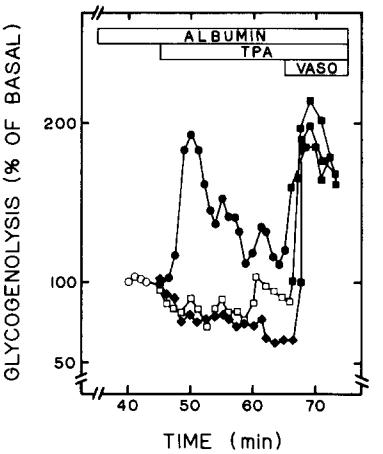


Figure 1. EFFECT OF TPA, VASOPRESSIN AND METABOLIC INHIBITORS ON GLYCOGENOLYSIS.

The concentration of the agents was as follows: TPA, 10 ng/ml (●) vasopressin 10 mU/ml (■), INDO 100 μM (□) and BPB 100 μM (◆). The results are the means of 4-6 experiments and are plotted as percentage of basal glucose output, which was 70 ± 5 μmol $g^{-1}hr^{-1}$.

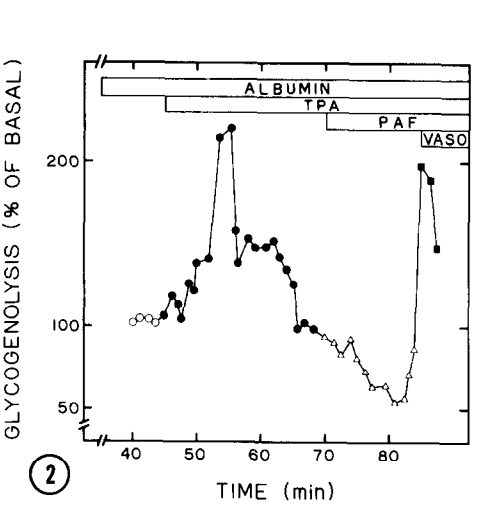
of vasopressin (10 mU/ml) to the medium resulted in a prompt stimulation of glycogenolysis (Fig. 1).

As shown in Fig. 1, the effect of TPA was blocked by the presence of 100 μM INDO (an inhibitor of cyclooxygenase) or 100 μM BPB (an inhibitor of phospholipase A_2). In contrast the effect of vasopressin was not affected by these inhibitors (Fig. 1).

In order to gain further insight on the mechanism of action of TPA the effect of subsequent addition of maximally effective concentrations of TPA (10 ng/ml) and PAF (10^{-7}M) and vice-versa on glycogenolysis were studied.

As previously mentioned, TPA produced a transient stimulation of glycogenolysis (Figs. 1 and 2); once its effect diminished, PAF was added, but no increase in glucose production was observed (Fig. 2). In contrast addition of vasopressin (in the presence of TPA and PAF) resulted in an immediate increase in hepatic glucose output (Fig. 2).

When PAF was added to the perfusion medium as first agent, a clear but transient stimulation of glycogenolysis was produced (Fig. 3). Addition of TPA, after the action of PAF had declined, resulted in no increase in glucose



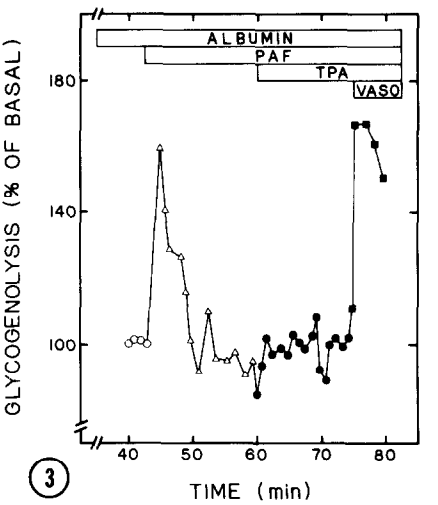


Figure 2. EFFECT OF SEQUENTIAL PERFUSION WITH TPA, PAF and VASOPRESSIN OR GLYCOGENOLYSIS.
The concentration of the agents was as follows: TPA 10 ng/ml (●), PAF 10^{-7}M (△), vasopressin 10 mU/ml (■). Other indications as in Fig.1

Figure 3. EFFECT OF SEQUENTIAL PERFUSION WITH PAF (△), TPA (●) AND VASOPRESSIN (■) ON GLYCOGENOLYSIS. Other indications as in Figure 1,2.

production. Again, the subsequent addition of vasopressin clearly stimulated glycogenolysis (Fig. 3).

DISCUSSION

The present data confirm the ability of phorbol esters to stimulate glycogenolysis in perfused liver. Several similarities exist between the actions of TPA and those of PAF on liver metabolism. Firstly, both agents are able to stimulate glycogenolysis in perfused liver (7-12 and present study) but not in isolated hepatocytes (4-6, 10, 12). The most likely explanation for these discrepancy is that cells other than hepatocytes (i.e. kupfter cells, muscle cells or endothelial cells) are directly affected by the agents, and that such action results in the release of a mediator which alters hepatocyte metabolism (10, 12).

We have previously suggested a role for cyclooxygenase products as mediators of the action of PAF (12). A similar suggestion can be made for the effect of TPA on the basis of the ability of INDO and BPB to block it. It is interesting to note that continuous perfusion (12) or sequential addition of

PAF (8, 9) results in desensitization to PAF but not to other agents (12). The data can be interpreted as the result of depletion of the putative mediator or of a metabolic precursor. The response to TPA in perfused liver also desensitizes rather rapidly. Furthermore, once the response to any of these agents (PAF or TPA) vanished no response to the addition of the other is observed under conditions where the liver retains full ability to increase glucose output in response to vasopressin. The data further support the suggestion that a common pathway (generation of a cyclooxygenase product) is involved in the action of both agents.

It is important to note that it was recently reported that liver perfusion with aggregated immunoglobins also results in an INDO-sensitive stimulation of glycogenolysis (11). Thus, several agents seem to be capable of indirectly stimulating hepatocyte metabolism through the generation of cyclooxygenase products. This may have physiological importance under normal or pathological (shock, inflamation or anaphylaxis) conditions.

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