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Activation of protein kinase C inhibits hormonal stimulation of the GTPase activity of Gi in human platelets

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The effect of 12-tetradecanoyl phorbol 13-acetate on the GTPase activity of Gi was investigated. Treatment with TPA did not alter basal GTPase activity of membranes or the stimulatory effect of prostaglandin E_1 (putatively via Gs). In contrast, the phorbol ester markedly diminished stimulation of GTPase by agents whose receptors are coupled to Gi such as epinephrine (α_2-adrenergic action), platelet activating factor or thrombin. Pertussis toxin catalyzed ADP-ribosylation was also decreased in membranes from TPA-treated platelets as compared to the controls. It is suggested that the alteration in the hormonal activation of the GTPase activity of Gi is secondary to a perturbation in the receptor-Gi interaction.

Protein kinase C; Enzyme activation; Gi; GTPase activity; α_2-Adrenoceptor

1. INTRODUCTION

There is evidence that protein kinase C (PKC) can modulate the function of signalling elements such as some receptor, G-proteins and membrane effectors (adenylate cyclase, phospholipase C and some ion channels) [1]. In platelets it has been observed that activation of PKC by 12-tetradecanoyl phorbol 13-acetate (TPA) largely impairs the GTP-dependent hormone-sensitive inhibitory pathway to adenylate cyclase which involves the inhibitory GTP-binding protein, Gi [2–6]. It has been shown that PKC phosphorylates Gi [3] and blocks its function in hormonal inhibition of adenylate cyclase. Interestingly, the ability of hydrolysis-resistant GTP analogues to inhibit adenylate cyclase is not altered by the treatment with TPA [5,6] which suggests that the receptor/Gi interaction rather than the Gi/adenylate cyclase interaction is what is altered by PKC. Further support for this interpretation was obtained in binding studies [6]; it was observed that in control platelets, approximately 75% of the receptors were in a high-affinity state for epinephrine and approximately 25% in a low-affinity state; Gpp(NH)p shifted the receptor affinity towards the low-affinity conformation [6]. In membranes from TPA-treated platelets, the receptors were in the low-affinity state and no further decrease in affinity was induced by Gpp(NH)p [6]. We examined the effect of TPA on hormone-stimulated GTPase activity; our results indicate that stimulation of PKC markedly diminished the hormone-stimulated GTPase activity of Gi.

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2. MATERIALS AND METHODS

Blood was obtained from healthy men and women who had taken no medication during the previous 2 weeks. Platelet-rich plasma was obtained by centrifugation. After a precquilibration period of 5 min at 37°C, the platelets were challenged with 1 μM TPA, or vehicle for 1 min; the platelets were centrifuged and homogenized. A crude membrane preparation was obtained as described by Hoffman et al. [7].

GTPase activity was assayed according to Cassel and Selinger [8], in a mixture containing 2 mM $MgCl_2$, 1 mM 3-isobutyl-1-methylxanthine, 0.1 mM cyclic AMP, 5 mM creatine phosphate, 1.2 mg/ml creatine kinase, 0.2% bovine serum albumin, 1 mM dithiothreitol, 0.1 mM EDTA, 1 μM GTP, 50 mM triethanolamine-HCl, pH 7.4, and [γ^{32}P]GTP (0.2 μCi/tube). The reaction was initiated by the addition of the membranes (10 μg of protein) and was carried out for 10 min or the times indicated at 25°C in a final volume of 0.1 ml. The release of [^{32}P]P_i was determined as described by Aktories and Jakobs [9]. Protein was quantified by the method of Lowry et al. [10]. ADP-ribosylation was assayed in a mixture containing 250 mM potassium phosphate buffer pH 7.5, 10 mM arginine, 5 mM $MgCl_2$, 1 mM ATP, 10 mM thymidine, 0.75 mM NADP, 0.1 mM GTP, 10 μM NAD and [^{32}P]NAD (10 μCi/tube). The reaction was carried out for 60 min at 30°C for 1 h. Pertussis toxin was activated with 20 mM DTT for 10 min at 37°C. The reaction was started by the addition of the membranes (100 μg); following the reaction, 1 ml of phosphate buffer was added and the membranes were pelleted by centrifugation. The pellet was dissolved and subjected to SDS-PAGE. The gels were fixed, dried and exposed to X-ray film at −72°C [11].

Immunoprecipitation of Giα was achieved using a specific polyclonal antibody generated against the decapeptide corresponding to a Gi (KNNLKDCGLF) [13], essentially as described for Gsα [12].

3. RESULTS

Under the conditions employed, basal GTPase activity was linear as a function of time of incubation and of similar magnitude in membranes from control and TPA-treated platelets (Fig. 1). Epinephrine (100 μM) and prostaglandin E_1 (PGE_1) (1 μM) increased the rate of GTPase activity (Fig. 1). The effect of epinephrine

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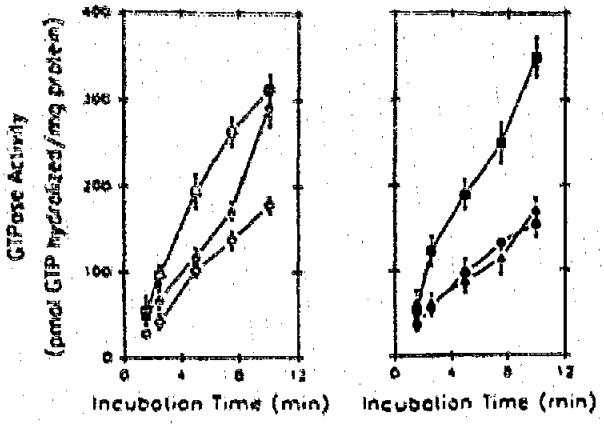

Fig. 1. GTPase activity of membranes from control and TPA-treated platelets. Membranes were incubated in the absence of any agent (circles) or in the presence of 100 μM epinephrine (triangles) or 1 μM PGE (squares). (Left panel) Open symbols = control membranes; (right panel) closed symbols = membranes from TPA-treated cells. Plotted are the means and vertical lines representing the SEM of 4 experiments performed in triplicate.

was blocked by yohimbine but not by prazosin indicating the involvement of α_2-adrenoceptors (data not shown). The effect of PGE_1 was similar in magnitude in membranes from control or TPA-treated platelets but, in contrast, the action of epinephrine was greatly diminished in membranes from TPA-treated cells as compared to the controls (Fig. 1). Dose/response curves (Fig. 2) confirmed this finding; i.e. PGE_1 stimulated similarly and in a dose-dependent fashion the GTPase activity of membranes from control and TPA-treated platelets; epinephrine induced a dose-dependent GTPase activation in control membranes

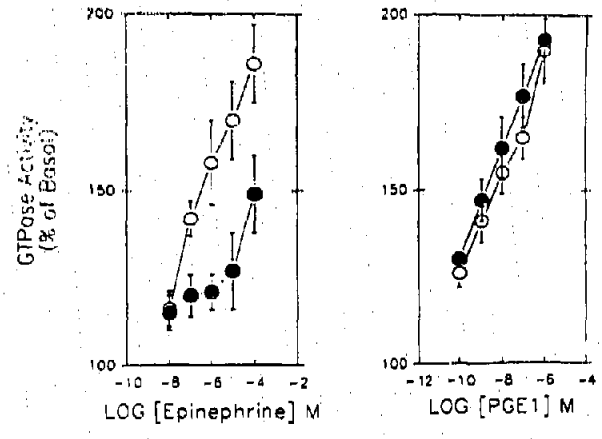

Fig. 2. Effect of TPA on the stimulations of GTPase activity induced by epinephrine or prostaglandin E_1. Membranes from control (open circles) or TPA-treated platelets (closed symbols) were incubated for 10 min with different concentrations of epinephrine or PGE_1. Results are presented as % of basal activities that were 18 ± 3 and 15 ± 3 pmol · min^{-1} · mg $protein^{-1}$ for membranes from control and TPA-treated cells, respectively. Plotted are the means and vertical lines represent the SE of 10 experiments performed in triplicate.

Table I

Effects of epinephrine, prostaglandin E_1, thrombin and platelet activating factor (PAF) on the GTPase activity of membranes from control and TPA-treated platelets

Agent	Control	TPA
	Percentage of basal activity	
Epinephrine (100 μM)	172 ± 7	144 ± 7[a]
PGE_1 (1 μM)	191 ± 10	198 ± 8
PAF (100 nM)	145 ± 7	124 ± 4[b]
Thrombin (1 U/ml)	141 ± 8	120 ± 3[b]

Results are the means ± SE of 4 experiments performed in triplicate. Basal values are the same as in Fig. 2.
[a] $P < 0.025$
[b] $P < 0.05$

but only increased GTPase activity at the highest concentration tested (100 μM) in membranes from TPA-treated cells (Fig. 2).

The decreased activation of GTPase activity was not exclusive to epinephrine but common to other agents whose receptors interact with Gi, such as platelet activating factor and thrombin (Table I).

Pertussis toxin-catalyzed ADP-ribosylation of membranes from control and TPA-treated platelets showed the labeling of ~41 kDa protein(s). Interestingly, in

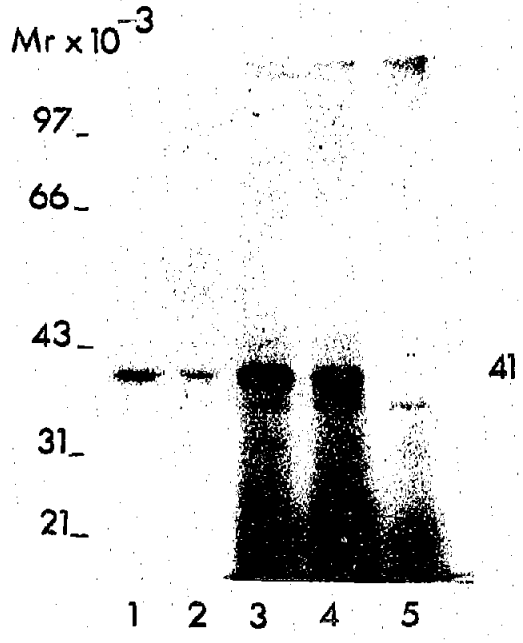

Fig. 3. Effect of TPA on the pertussis toxin catalyzed ADP-ribosylation in membranes from control and TPA-treated platelets. Membranes from control (lines 1, 3, 5) or TPA-treated platelets (lines 2, 4) were incubated under the conditions for [^{32}P]ADP-ribosylation in the absence (line 5) or presence (lines 1-4) of pertussis toxin. The membranes were solubilized and either subjected to SDS-PAGE (lines 3-5) or immunoprecipitated using a Gi-specific antibody and the precipitate subjected to SDS-PAGE (lines 1, 2). The autoradiogram is representative of 5, all with similar results.

membranes from TPA-treated platelets the labeling was consistently decreased 30-50% (Fig. 3). The labeled protein was immunoprecipitated using an specific anti-Gi antiserum (Fig. 3); in the immunoprecipitation the decrease in labeling induced by the treatment with TPA was even clearer (Fig. 3).

4. DISCUSSION

The findings that activation of PKC in platelets blocks the hormonal inhibition of adenylate cyclase activity [2-6] but not that induced by Gpp(NH)p [5,6] indicated that the receptor/Gi interactions rather than the Gi/cyclase interactions were altered [6]. Further support for this interpretation was obtained in the binding studies [6]. Our current data are consistent with such an interpretation; they clearly show that the effect of agents whose receptors activate the GTPase activity of Gi is markedly decreased. In contrast, the stimulation of GTPase activity induced by PGE_1 (putatively via activation of Gs) is not altered which is also consistent with the absence of effect of TPA on the activatory branch of adenylate cyclase [2-6].

We did not observe any effect of the treatment with TPA on the basal GTPase activity. However, 'basal' GTPase activity probably is the result of GTP hydrolysis by several G-proteins. Therefore, we cannot rule out a direct effect of the treatment on the hydrolytic activity of Gi. Nevertheless, a blockade of the GTPase activity would probably result in a tonic activation of this G-protein (as observed in Gs, ADP-ribosylated by cholera toxin [14]); this is not what is observed.

Current ideas indicate that the hormonal activation of the GTPase activity of G-proteins is probably secondary to the stimulation of the GDP/GTP exchange promoted by their interaction with activated receptors [15]. The perturbation of the receptor/Gi interaction [6] by the treatment with TPA could be responsible for the blockade of the hormonal inhibition of adenylate cyclase [2-6] and activation of the GTPase activity of Gi.

It has been suggested that the C-termini of Gα subunits govern in part the interaction of G-proteins with receptors [16]. Using a panel of specific antibodies against this domain, Simonds et al. [13] were able to block the hormonal inhibition of adenylate cyclase and identified the G-protein involved in this effect as Gi2. Interestingly, the consensus site for pertussis toxin-catalyzed ADP-ribosylation is located in the same domain [17]. It is possible that the decreased labeling observed by us with TPA and previously by others using other agents [18] could be due, at least partially, to a perturbation of the C-terminus (receptor-coupling domain) of Gi2. Other possibilities such as G-protein subunit dissociation, cannot be ruled out.

The molecular basis of the alteration of Gi2 function remains to be determined. Although in vitro experiments suggested that PKC phosphorylates Gi [3], recent evidence indicates that phorbol esters phosphorylate a G-protein other than Gi [19]. It is interesting to note that it has been shown that TPA blocks Gi function in liver cells [20] and that this effect is associated with the phosphorylation of Gi α [20]; however, the blockade of Gi function observed in liver cells is different to that observed in platelets, i.e. the inhibitory action of hydrolysis-resistant analogues of GTP is blocked [20].

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