Staurosporine inhibits the respiratory burst and induces exocytosis in human neutrophils

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The protein kinase C inhibitor staurosporine influenced in different ways the functions of human neutrophils. Staurosporine prevented the enhanced protein phosphorylation in phorbol ester- and *N*-formylmethionyl-leucylphenylalanine (fMLP)-stimulated cells, and was a powerful inhibitor of the respiratory burst induced by phorbol myristate acetate [IC_{50} (concentration causing 50 % inhibition) 17 nM] and the chemotactic peptides fMLP and C5a (IC_{50} 24 nM). It did not alter, however, the superoxide production by cell-free preparations of NADPH oxidase. Staurosporine had no effect on agonist-dependent changes in cytosolic free Ca^{2+} and exocytosis of specific and azurophil granules, and showed only a slight inhibition of the release of vitamin B_{12} -binding protein induced by phorbol myristate acetate (decreased by 40 % at 200 nM). On the other hand, staurosporine also exhibited neutrophil-activating properties: it induced the release of gelatinase (from secretory vesicles) and vitamin- B_{12} -binding protein (from specific granules). These effects were protracted, concentration-dependent, insensitive to Ca^{2+} depletion, and strongly enhanced by cytochalasin B. Staurosporine, however, did not induce the release of β -glucuronidase or elastase (from azurophil granules). Except for the sensitivity to cytochalasin B, these properties suggest a similarity between the exocytosis-inducing actions of staurosporine and PMA. The results obtained with staurosporine provide further evidence that different signal-transduction processes are involved in neutrophil activation, and suggest that protein phosphorylation is required for the induction of the respiratory burst, but not for exocytosis.

INTRODUCTION

It is presumed that protein kinase C (PKC) becomes activated when neutrophils are stimulated with phorbol esters or chemotactic agonists (Berridge, 1986). The role of PKC in signal transduction and the induction of different neutrophil responses, however, is still disputed. Various types of protein kinase inhibitors, e.g. isoquinoline sulphonamides (Hidaka *et al.*, 1984; Gerard *et al.*, 1986) and sphingoid long-chain bases (Hannun *et al.*, 1986) have been used in different laboratories to study this question. We have now assessed the effects of staurosporine, a microbial alkaloid with anti-fungal properties (Tamaoki *et al.*, 1986), which was recently reported to inhibit PKC at nanomolar concentrations (Nakadate *et al.*, 1988). Staurosporine showed a dual effect. It acted as an inhibitor of the respiratory burst and protein phosphorylation, and as a stimulus of exocytosis of specific granules and secretory vesicles. These results suggest that different signal-transduction processes are involved in neutrophil activation and that PKC-dependent protein phosphorylation is required for the respiratory burst but not for exocytosis.

EXPERIMENTAL

Materials

N-Formylmethionyl-leucylphenylalanine (fMLP), platelet-activating factor (PAF) and *N*-methoxysuccinyl-

Ala-Ala-Pro-Val-7-amido-4-methylcoumarin were obtained from Bachem A.G., Bubendorf, Switzerland; phorbol myristate acetate (PMA) and cytochrome *c* (type III from horse heart) from Sigma, St Louis, MO, U.S.A.; cytochalasin B and soybean trypsin inhibitor from Serva G.m.b.H., Heidelberg, Germany; staurosporine and bovine serum albumin (BSA) from Fluka A.G., Buchs, Switzerland; quin-2/AM from Calbiochem A.G., Luzern, Switzerland; Lympho-paque from Nyegaard and Co., Oslo, Norway; ^{32}P -labelled orthophosphate (in aqueous solution, HCl-free, carrier-free) from Amersham, Bucks., U.K.; human recombinant neutrophil-activating factor (NAF) was obtained from the Sandoz Research Institute, Vienna, Austria, and the chemotactic peptide C5a was kindly provided by Dr C.A. Dahinden, Department of Clinical Immunology, University of Bern, Bern, Switzerland. The medium, adjusted to pH 7.4, consisted of 137 mM-NaCl, 2.7 mM-KCl, 8.1 mM- Na_2HPO_4 , 1.5 mM- KH_2PO_4 , 0.9 mM- $CaCl_2$, 0.49 mM- $MgCl_2$ and BSA (2.5 mg/ml) [phosphate-buffered saline (PBS)/BSA], except where stated otherwise.

Cells

Human neutrophils were isolated from fresh donor blood as described previously (Dewald & Baggiolini, 1986). The final cell suspension (10^8 cells/ml) in 154 mM-NaCl supplemented with 0.05 mM- $CaCl_2$ contained on average 98 % granulocytes.

Abbreviations used: PMA, phorbol 12-myristate 13-acetate; fMLP, *N*-formylmethionyl-leucylphenylalanine; PAF, platelet-activating factor; PKC, protein kinase C; BSA, bovine serum albumin; NAF, neutrophil-activating factor; PBS, phosphate-buffered saline (see the text for composition and pH); IC_{50} , concentration causing 50 % inhibition.

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Respiratory burst

Superoxide formation was measured in a Hewlett-Packard 8451A diode array spectrophotometer as described by Thelen *et al.* (1988a). Neutrophils (2×10^6 /ml) were preincubated in plastic cuvettes for 5 min at 37 °C in the presence of 85 μ M-cytochrome *c*, and the reaction was started by adding staurosporine and the appropriate stimulus at the indicated interval. Cell-free NADPH oxidase was prepared from control and PMA-stimulated cells (Markert *et al.*, 1984).

Exocytosis

Standard methods (Dewald & Baggiolini, 1986) were used with minor modifications. Briefly, neutrophils (4×10^6 /ml) suspended in PBS/BSA were preincubated for 5 min at 37 °C with or without cytochalasin B (5 μ g/ml) before addition of the appropriate stimulus. After the indicated time the reaction was stopped by rapid cooling to 0 °C, followed by centrifugation. Markers were determined in the cell-free media and cell pellets. Lactate dehydrogenase, gelatinase, vitamin B₁₂-binding protein and β -glucuronidase were assayed as described by Dewald & Baggiolini (1986). Elastase was measured in 0.1 ml samples of the cell-free media using *N*-methoxy-succinyl-Ala-Ala-Pro-Val-7-amido-4-methylcoumarin as substrate (Dewald & Baggiolini, 1987). In the experiments where vitamin B₁₂-binding protein was measured, soybean trypsin inhibitor (50 μ g/ml) was added to the incubation medium to prevent proteolytic degradation.

Cytosolic free Ca²⁺ measurements

Quin-2 loading was performed by incubating 0.1 nmol of quin-2 acetoxymethyl ester/ 10^6 cells for 15 min at 37 °C. After centrifugation the loaded cells were resuspended in medium containing 1 mM-CaCl₂. Changes in the cytosolic free Ca²⁺ concentration were determined as described previously (von Tschärner *et al.*, 1986).

Protein phosphorylation

Neutrophils were loaded with ³²P essentially as described by Okamura *et al.* (1988a). Briefly, neutrophils (3×10^7 /ml) washed and suspended in 20 mM-Hepes/Tris, pH 7.4, containing 150 mM-NaCl, 5 mM-KCl and 10 mM-glucose, were added to an equal volume of ³²P-labelled orthophosphate (4 mCi/ml) in the same buffer, and incubated for 1 h at 37 °C. The cells were then diluted 2-fold with ice-cold incubation buffer, centrifuged, washed twice and finally suspended at 6×10^6 cells/ml in the same buffer supplemented with 1 mM-MgCl₂ and 0.25 mM-CaCl₂. For activation, 0.5 ml of the ³²P-loaded neutrophils were prewarmed to 37 °C, treated where indicated with staurosporine (100 nM), and stimulated 2 min later with 1 μ M-fMLP for 45 s or 300 nM-PMA for 2 min. The reaction was terminated by addition of 0.5 ml of 20% (w/v) trichloroacetic acid. After 45 min on ice the samples were centrifuged, the pellets washed with 5% trichloroacetic acid and prepared for two-dimensional gel electrophoresis according to the instruction manual supplied with the Mini-PROTEAN II 2-D cell from Bio-Rad Laboratories.

RESULTS

Respiratory burst

Staurosporine inhibited the respiratory burst elicited by PMA and fMLP. The effect on the two responses was

Fig. 1. Effect of staurosporine on the maximum rate of superoxide production during the respiratory burst induced by fMLP and PMA

(a) Dependence on the concentration of staurosporine added to the neutrophils 2 min before stimulation with 100 nM-fMLP (Δ) or 30 nM-PMA ($\square$). (b) Dependence on the staurosporine preincubation time. The abscissa indicates the time interval between addition of 50 nM-staurosporine and stimulation with 100 nM-fMLP (Δ) or 30 nM-PMA ($\square$). Values are means obtained with 3–11 different neutrophil preparations.

concentration-dependent but not identical, as indicated by the different slopes of the inhibition curves (Fig. 1a) and the small, but significant, difference ($P=0.005$) in the IC_{50} (concentration causing 50% inhibition) values which amounted to 17 ± 2 nM ($n=9$) for PMA and 24 ± 7 nM ($n=11$) for fMLP. In three experiments, the IC_{50} for the respiratory burst induced by C5a, another chemotactic agonist, was 23 ± 6 nM, which is indistinguishable from the value obtained for fMLP. Staurosporine also inhibited the enhanced respiratory burst that is observed when neutrophils are primed with PAF and stimulated with fMLP (Baggiolini *et al.*, 1988) (results not shown). In all these experiments staurosporine was added to the neutrophils 2 min before the stimulus. Variation of this interval markedly affected the inhibition of the response to fMLP. By contrast, inhibition of the respiratory burst induced by PMA was not time-dependent and was already maximal when stimulus and inhibitor were applied together (Fig. 1b). As illustrated in Fig. 2, a rapid and concentration-dependent inhibition of superoxide production was also observed when staurosporine was added after PMA. On the other hand, staurosporine did not affect the NADPH-dependent production of superoxide by cell-free oxidase prepared from PMA-stimulated neutrophils. These observations indicate that staurosporine does not inhibit the NADPH-oxidase itself, and that its effect on the respiratory burst of intact cells presumably results from an interference with signal transduction.

Exocytosis

The study of exocytosis revealed that staurosporine has neutrophil-stimulating activity. As Table 1 shows, staurosporine induced the release of vitamin B₁₂-binding protein from the specific granules like fMLP and PMA. Its effect was moderate in normal cells (about 50 and 20% of the release induced by fMLP and PMA respectively), but became very pronounced when the cells were pretreated with cytochalasin B. Under these conditions, significant

release was already apparent at a staurosporine concentration as low as 10 nM. Interestingly, an enhanced response was also obtained when cytochalasin B was added 5 min after staurosporine. Cytochalasin B does not appreciably modify the response to PMA (Di Virgilio *et al.*, 1984) and enhances the effect of fMLP and other agonists only when applied before the stimulus.

Staurosporine did not induce exocytosis of azurophilic granules, as assessed by β -glucuronidase release. It differed in this respect from fMLP, which showed release provided that the cells were pretreated with cytochalasin B (Table 1). These results, and the lack of liberation of lactate dehydrogenase, indicate that staurosporine was not cytolytic.

As Fig. 3 shows, vitamin B₁₂-binding protein was released together with gelatinase, indicating that staurosporine induced the discharge of secretory vesicles as well. The response was slow and protracted, similar to

Fig. 2. Inhibition of an ongoing PMA-induced respiratory burst by staurosporine

Neutrophils were stimulated with 30 nM-PMA and then exposed to staurosporine at the concentrations indicated. Staurosporine was added 3 min after PMA, as indicated by the horizontal break in the curves. Original cytochrome *c* reduction recordings from one of four similar experiments are shown.

that observed with PMA, and clearly different from the rapid transient effect of fMLP. Partial Ca²⁺-depletion of the neutrophils, obtained by preincubation for 90 min with 1 mM-EGTA in the absence of Ca²⁺, did not influence exocytosis induced by staurosporine or PMA, but reduced the response to fMLP by 60–70 %.

Possible effects of staurosporine on PMA- and fMLP-induced exocytosis were also studied (Table 2). The fMLP-dependent release of vitamin-B₁₂-binding protein was not affected by staurosporine in normal neutrophils. It was enhanced in cells that had been treated with cytochalasin B, conditions under which staurosporine alone induced appreciable release. The release induced by PMA was slightly inhibited at high concentrations of

Fig. 3. Time course of release of gelatinase (a, b) and vitamin B₁₂-binding protein (c, d)

Neutrophils were preincubated in the absence (a, c) or presence (b, d) of cytochalasin B and then stimulated with 10 ($\diamond$), 30 ($\square$) or 100 nM ($\blacksquare$)-staurosporine, 10 nM-PMA ($\circ$) or 100 nM-fMLP ($\bullet$). $\blacktriangle$, Release by unstimulated control cells. Results from one representative experiment are shown.

Table 1. Effect of staurosporine on exocytosis

Neutrophils were preincubated with or without cytochalasin B (CB), stimulated as indicated and incubated for 20 min. Values are means $\pm$ S.D. Further abbreviations: LDH, lactate dehydrogenase; stauro, staurosporine; Vit.B₁₂-BP; vitamin B₁₂-binding protein. *n* is the number of different neutrophil preparations used.

Stimulus	Concn. (nM)	CB	Release (%)			<i>n</i>
			Vit.B ₁₂ -BP	β -Glucuronidase	LDH	
None		—	4.5 $\pm$ 2.2	2.2 $\pm$ 0.9	2.7 $\pm$ 1.2	5
Stauro	100	—	12.8 $\pm$ 3.7	2.5 $\pm$ 0.2	2.1 $\pm$ 0.6	3
fMLP	100	—	21.0 $\pm$ 2.1	4.8 $\pm$ 1.3	2.2 $\pm$ 0.9	3
PMA	10	—	59.8 $\pm$ 2.9	3.5 $\pm$ 1.6	5.3 $\pm$ 1.6	3
None		+	7.1 $\pm$ 3.5	2.3 $\pm$ 0.7	2.4 $\pm$ 1.5	5
Stauro	10	+	27.7 $\pm$ 3.6	1.7 $\pm$ 1.5	3.1 $\pm$ 1.3	3
	30	+	56.1 $\pm$ 5.5	1.9 $\pm$ 1.1	2.8 $\pm$ 2.2	3
	100	+	66.7 $\pm$ 6.9	2.9 $\pm$ 1.3	2.7 $\pm$ 1.4	5
fMLP	100	+	75.0 $\pm$ 7.4	33.1 $\pm$ 4.2	2.1 $\pm$ 1.4	5

Table 2. Effect of staurosporine on fMLP- and PMA-induced release of vitamin-B₁₂-binding protein

To neutrophils preincubated with or without cytochalasin B (CB), staurosporine was added at the appropriate concentration and, 2 min later, fMLP or PMA was added as indicated. Incubation was then continued for 10 min. Values represent the release as a percentage of the initial cellular content. Results are from one of three closely corresponding experiments.

Stimulus	Concn. (nM)	CB	[Staurosporine] (nM)...	Release (%)				
				0	10	30	100	200
None		—		3.9	5.0	4.2	6.5	8.2
fMLP	100	—		23.8	21.2	18.9	23.6	24.6
PMA	30	—		49.3	49.9	50.0	38.2	30.1
None		+		5.9	6.3	20.2	42.3	32.9
fMLP	100	+		77.8	71.8	79.2	84.7	92.1

Fig. 4. Effect of staurosporine on agonist-induced release of azurophil-granule enzymes

To neutrophils pretreated with cytochalasin B, staurosporine was added at the indicated concentrations. After 2 min the cells were stimulated with 100 nM-fMLP (○), 1 nM-C5a (□), 10 nM-NAF (◇) or 1 μM-PAF (△), and the activities of β-glucuronidase (a) and elastase (b) released within 10 min were determined. Results of one of three similar experiments are shown.

staurosporine (average inhibition of 22 % and 41 % at 100 and 200 nM respectively in four experiments). As Fig. 4 shows, staurosporine had no effect on the release of β-glucuronidase and elastase from the azurophil granules when cytochalasin B-pretreated neutrophils were stimulated with different chemotactic receptor agonists.

Cytosolic free Ca²⁺

A rise in cytosolic free Ca²⁺ is an essential signal-transduction event in agonist-stimulated neutrophils. As Fig. 5 shows, Ca²⁺-associated fluorescence changes in response to fMLP were not modified by staurosporine in either normal or cytochalasin B-treated neutrophils. Staurosporine itself did not influence the cytosolic free Ca²⁺ level (results not shown). For these experiments staurosporine was used at 100 nM, a concentration that induced significant exocytosis of vitamin B₁₂-binding protein and gelatinase (Fig. 3) and inhibited the fMLP- and PMA-elicited respiratory burst by 80 and 100 % respectively (Fig. 1).

Protein phosphorylation

Loading of neutrophils with ³²P revealed the phosphorylation of a number of cellular proteins as detected

Fig. 5. Effect of staurosporine on agonist-induced changes in cytosolic free Ca²⁺

Neutrophils preincubated for 90 s in the absence (a) or presence (b) of cytochalasin B were treated with or without 100 nM-staurosporine for another 90 s and then stimulated with 10 nM-fMLP. Each panel shows tracings obtained with and without staurosporine. A slight elevation in cytosolic free Ca²⁺ is known to occur upon cytochalasin B treatment (Treves *et al.*, 1987) and explains the higher pre-stimulation levels in (b). Original recordings of quin-2-associated fluorescence obtained in a representative experiment are shown.

by two-dimensional gel electrophoresis (Fig. 6a). Upon stimulation with either PMA (Fig. 6b) or fMLP (Fig. 6c), phosphorylation of the 48 kDa protein and several other proteins in the 40–80 kDa range became more prominent, and additional phosphorylated proteins in the same molecular-mass range appeared. Treatment with staurosporine did not appreciably change the phosphorylation pattern of resting cells (Fig. 6d), but prevented the enhanced phosphorylation of the 40–80 kDa proteins induced by PMA (Fig. 6e) or fMLP (Fig. 6f). These results provide evidence that a staurosporine-sensitive protein kinase is activated by PMA and fMLP.

DISCUSSION

Staurosporine has been shown to inhibit PKC from several cellular sources (Tamaoki *et al.*, 1986; Kiyoto *et al.*, 1987; Nakadate *et al.*, 1988; Vegesna *et al.*, 1988; Wolf & Baggiolini, 1988) and to act on both the cytosolic and membrane-associated form of the enzyme obtained from mouse epidermal cells (Kiyoto *et al.*, 1987). Using

Fig. 6. Inhibition of stimulus-dependent protein phosphorylation by staurosporine

Neutrophils ($6 \times 10^6/\text{ml}$) were incubated for 2 min at 37°C with or without 100 nM-staurosporine (lower and upper panels respectively) and then exposed to solvent buffer (a, d) or stimulated with 300 nM-PMA for 2 min (b, e) or $1 \mu\text{M}$ -fMLP for 45 s (c, f). Autoradiographs show phosphoproteins from 1.5×10^5 cells separated by two-dimensional SDS/polyacrylamide-gel electrophoresis (the basic region of the gel is on the left). Results from one of three similar experiments are shown.

PKC purified from mouse brain cytosol, Nakadate *et al.* (1988) showed that staurosporine inhibits the proteolytically generated catalytic domain of PKC without affecting phorbol ester binding. Furthermore, staurosporine, like phorbol esters, was found to induce the association of purified PKC with human erythrocyte membranes and to inhibit the membrane-associated enzyme (Wolf & Baggiolini, 1988). Staurosporine, however, is not selective for PKC. It also inhibits cyclic AMP- and cyclic GMP-dependent protein kinases, but its potency toward PKC is about 10-fold higher (Tamaoki *et al.*, 1986).

The present study shows that staurosporine prevents stimulus-dependent protein phosphorylation in intact neutrophils and the activation of the respiratory burst without appreciably inhibiting other expressions of receptor-mediated activation such as cytosolic free Ca^{2+} changes and exocytosis. On the other hand, staurosporine appears to have activating properties as well, as indicated by the induction of exocytosis from the specific granules and secretory vesicles.

Inhibition of the PMA-elicited respiratory burst by staurosporine was previously reported by Sako *et al.* (1988). In contrast with the findings of these authors, we found that staurosporine inhibits the respiratory-burst response to the chemotactic peptides fMLP, C5a and NAF (Thelen *et al.*, 1988b) as well. The discrepancy may be explained by the experimental conditions, since Sako *et al.* (1988) used cytochalasin B-treated neutrophils and fMLP at 10–50-fold the maximum effective concentration. Inhibition of the response to both stimuli was also observed with sphinganine (Wilson *et al.*, 1986; Lambeth *et al.*, 1988). The results obtained with isoquinoline sulphonamides like H-7 or C-I are difficult to interpret, mainly because of the low potency of these compounds. Even at $100 \mu\text{M}$, 1000-fold the fully inhibitory concentration of staurosporine, the isoquinoline

sulphonamides only partially inhibit the PMA-induced respiratory burst (Bass *et al.*, 1987; Berkow *et al.*, 1987; Salzer *et al.*, 1987; Seifert & Schaechtele, 1988; Sha'afi *et al.*, 1988). The effects on the response to fMLP are controversial, since inhibition (Fujita *et al.*, 1986; Nath *et al.*, 1989), as well as no effect (Gerard *et al.*, 1986; Bass *et al.*, 1987; Berkow *et al.*, 1987; Sha'afi *et al.*, 1988), have been reported. Discrepancies may simply result from different experimental protocols, as suggested by our observation that the inhibition depended on the preincubation time when fMLP (in contrast with PMA) was the stimulus. Also, Nath *et al.* (1989) reported that the temperature at which H-7 or C-I is added to the cell suspension affects the inhibition of the fMLP- but not of the PMA-induced response.

The sensitivity to staurosporine suggests that the induction of the respiratory burst by receptor agonists or phorbol esters depends on a common signal-transduction step that is required for the generation of active NADPH oxidase. This process is likely to involve protein phosphorylation by PKC. Phosphorylation of a group of 48 kDa proteins upon stimulation of neutrophils with PMA or fMLP has been demonstrated by several groups (Heyworth & Segal, 1986; Okamura *et al.*, 1988a,b; Babior, 1988; Sha'afi *et al.*, 1988), and has been shown to be inhibited by H-7 (Sha'afi *et al.*, 1988) and by staurosporine (the present results). There is evidence that such phosphorylations are related to NADPH oxidase activation (Babior, 1988), and it is well known that neutrophils from patients with autosomal chronic granulomatous disease lack both the respiratory burst and phosphorylation of the 48 kDa proteins (Heyworth & Segal, 1986; Caldwell *et al.*, 1988; Okamura *et al.*, 1988a,b). A role for PKC in respiratory-burst activation, as indicated by the present data, is in agreement with recent evidence that two signal-transduction pathways, one dependent on, and the other independent of, Ca^{2+}

and PKC, are required for this response (Dewald *et al.*, 1988). The rapid discontinuation of superoxide production in PMA-stimulated cells by staurosporine suggests that the maintenance of the respiratory burst depends on the continuous activity and/or activation of PKC.

Staurosporine did not affect the rise in cytosolic free Ca^{2+} and receptor-mediated exocytosis of azurophil and specific granules. Even when exocytosis was induced with PMA, only a weak inhibitory effect was observed. The potency as inhibitor of the PMA-induced respiratory burst and release of vitamin- B_{12} -binding protein was clearly different (Fig. 1a and Table 2). These results are in agreement with previous findings obtained with H-7 or C-I (Berkow *et al.*, 1987; Salzer *et al.*, 1987; Sha'afi *et al.*, 1988) and confirm the conclusion that PKC activation plays only a minor role, if any, in the induction of neutrophil-granule release. Wilson *et al.* (1987), however, have reported that sphinganine inhibits the fMLP- and PMA-induced release from the specific, but not from the azurophil granules.

Unexpected was the finding that staurosporine induces exocytosis and thus behaves like a stimulus. In terms of time course and pattern of activity, staurosporine was similar to PMA. It induced the prolonged release of gelatinase and vitamin- B_{12} -binding protein (from secretory vesicles and specific granules respectively), but no release of azurophil-granule components, even in cytochalasin B-pretreated cells. The response to staurosporine (but not to PMA) was markedly enhanced by cytochalasin B, even when added after staurosporine, underlining the persistence of its action. The lack of azurophil-granule release, the extended time course of the response and its independence of cytosolic free Ca^{2+} changes clearly indicate that the mechanism of action of staurosporine differs from that of fMLP and other receptor agonists.

The similar properties of the release response induced by staurosporine and PMA suggest the possibility of a common step in the activation pathway leading to exocytosis from the specific granules and secretory vesicles which seems to be dissociated from PKC activity and protein phosphorylation, but could involve the non-catalytic subunit of PKC. This notion is supported by the recent finding that staurosporine and phorbol esters, despite their opposite effects on the catalytic function, promote the association of PKC with membranes (Wolf & Baggiolini, 1988). Recently PMA-like effects of staurosporine have also been observed in murine epidermal cells. The induction of ornithine decarboxylase by PMA was enhanced, rather than inhibited, by staurosporine, which also had by itself inducing effects (Kiyoto *et al.*, 1987). In addition, staurosporine led to changes in morphology and differentiation similar to those observed with phorbol esters (Sako *et al.*, 1988).

We thank Sabine Imer for pre-submission editorial assistance. This work was supported by Swiss National Science Foundation Grant 3.241-0.85.

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