Highly Lipophilic Phorbol Esters as Inhibitors of Specific [3H]Phorbol 12,13-Dibutyrate Binding

Nancy A. Sharkey and Peter M. Blumberg

ABSTRACT

We examined the ability of a series of highly lipophilic phorbol esters to inhibit [20-3H]phorbol 12,13-dibutyrate binding to the cytosolic aporeceptor from mouse brain. If added in the usual fashion directly into the aqueous phase of the assay mixture, phorbol 12,13-diestearate, phorbol 12,13-dioleate, and phorbol 12,13-dimyristate showed very weak inhibitory activities, with apparent inhibitor equilibrium dissociation constant values above 4 μM. In contrast, if incorporated directly into the liposomes used to reconstitute the aporeceptor, all three derivatives inhibited binding with high apparent affinities, 7.4 to 34 nM. The less lipophilic derivative phorbol 12,13-didodecanoate showed a similar high affinity, 2.4 to 3.2 nM, by either route of addition. Consistent with the activity of the lipophilic derivatives being masked by an inability to transfer from the aqueous to the lipid phase, phorbol 12,13-diarboxylic acid added to the aqueous phase inhibited efficiently (apparent inhibitor equilibrium dissociation constant, 14 nM) in the presence of 0.03% Triton X-100. The results suggest that the phorbol ester receptor recognizes phorbol esters which are incorporated into the lipid bilayer. They indicate, moreover, that the apparent low activity of the more lipophilic phorbol esters is strongly influenced by factors other than equilibrium binding affinities.

INTRODUCTION

The phorbol esters represent one of the most potent classes of mouse skin tumor promoters (24, 27). The compounds are of further interest because they exert profound effects on a variety of biological systems (4, 5, 14, 25). This laboratory and subsequently others have used [3H]PDBU to demonstrate the existence of specific receptors for the phorbol esters (see Ref. 6 for review). The close agreement in structure-activity relations between binding activity and potency for inducing biological responses strongly argues that these receptors mediate phorbol ester responses. Upon subcellular fractionation, the receptors are recovered partially in the particulate fraction and partially as an aporeceptor in the cytoplasmic fraction (1, 34, 35, 37). The aporeceptor requires addition of appropriate phospholipids for activity, and the binding properties of the lipid-protein complex depend markedly on the identity of the phospholipids used for the reconstitution (7). The cytoplasmic aporeceptor is the same as protein kinase C (1, 34, 35, 37). The membrane receptor is at least partially and may be entirely a protein kinase C-lipid complex (2, 7, 30).

Protein kinase C requires Ca2+ and phospholipids for enzymatic activity (see Ref. 40 for review). In the presence of limiting Ca2+ concentrations in vitro, protein kinase C is stimulated by diacylglycerol derivatives, which shift the Ca2+ dose-response curve to lower Ca2+ concentrations. Castagna et al. (10) have reported that phorbol esters stimulate protein kinase C in a fashion similar to diacylglycerols. In vivo, likewise, the diacylglycerol derivative glycerol 1-oleate 2-acetate and the phorbol esters induce similar, rapid biochemical responses in several systems (21, 29, 36, 40). Consistent with diacylglycerols being the postulated endogenous analogues of the phorbol esters, we have demonstrated that diacylglycerols inhibit phorbol ester binding competitively (39).

In the present study, we have analyzed the binding affinities of a series of highly lipophilic phorbol esters for the reconstituted aporeceptor from cytosol. The studies were designed to address 3 overlapping issues. The first issue was whether the phorbol ester receptor recognized phorbol ester which had partitioned into the membranes or that which remained free in aqueous solution. There were indirect arguments favoring both alternatives. On the one hand, biological potency is reduced for phorbol derivatives with fatty acid side chains of greater than optimal chain lengths. The greater effectiveness of PMA than of the more lipophilic derivative PDD is perhaps the most familiar example (3). Other examples include the homologous series of symmetrically substituted phorbol 12,13-diesters, among which phorbol 12,13-dioleate has greatest potency (41), and phorbol 12-acyl 13-acetate derivatives, among which the 12-myristate derivative is most potent (24). Since the more lipophilic derivatives would be expected to partition into membranes to a greater degree, their reduced biological activities suggested that the receptor recognized that decreasing fraction which remained free in aqueous solution. On the other hand, since diacylglycerol derivatives such as diolein are insoluble in aqueous solution, their ability to compete for phorbol ester binding argued that, in analogy, the phorbol esters present in the membranes should be those recognized. Determination of the potencies of phorbol esters sufficiently hydrophobic to have very low aqueous solubility might distinguish between these 2 alternatives.

A second motivation for the studies was to better understand the factors contributing to the phorbol ester structure-activity relations. For example, Scribner and Boutwell (38) had reported that PDBU and PDM were equally potent as tumor promoters. PDBU, however, was substantially more potent for causing mouse ear reddening. Did such discrepancies indicate separate receptor subclasses, as implied by those authors, or did they merely reflect pharmacokinetic distortion of the apparent PDM activity?
A third motivation was to better compare the relative potencies of diacylglycerol derivatives and of phorbol esters. Although we had reported earlier that the K, for diolen was 6 × 10^-7 M, that for PDBU, the relative contributions of the differing side chains and head groups could not be distinguished. Comparison of compounds possessing similar side chains would therefore be of interest.

MATERIALS AND METHODS

L-a-phosphatidyl-L-serine, PDBU, PDD, croton oil, and Trizma base were obtained from Sigma Chemical Co. (St. Louis, MO). [3H]PDBU (specific activity, 13.4 Ci/mmol), was purchased from New England Nuclear (Boston, MA). Phorbol was prepared from croton oil as described by Becker and Schmidt (27). Phorbol 12,13,20-tridecanoate, phorbol 12,13,20-tritetradecanoate, and phorbol 12,13,20-tristearate were prepared from phorbol and the appropriate acid chlorides as described by Bresch et al. (8), except that CHCl_3-pyridine (2:1, v/v) was used in place of pyridine. Phorbol 12,13,20-tritoleate was prepared in a similar manner using oleic anhydride and including N,N-dimethyl-4-aminopyridine as a catalyst. The phorbol 12,13-diesters were prepared from the phorbol 12,13,20-triesters by transesterification in methanol or CH_2Cl_2:methanol as described (8). Products were purified by high-pressure liquid chromatography on Altex Ultrasphere-Si columns using 2,2,4,4-timethylpentane:2-propanol as solvent systems.

[3H]PDBU binding was assayed using mouse brain cytosol, prepared as follows. The brains from 6- to 8-week-old female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) were removed, placed in an equal volume of 50 mM Tris-Cl, pH 9.0, 1 mM magnesium acetate, 1 mM phenylmethylsulfonyl fluoride, 2 mM ethylene glycol-bis-(beta-amino-ethyl ether)-N,N',N''-N'''-tetraacetic acid at 4°C, and homogenized in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 38,000 rpm for 60 min at 4°C in the 50 Ti rotor of a Beckman L8-70 ultracentrifuge. The supernatant was removed and recentrifuged for 45 min at 42,000 rpm. We refer to the supernatant from this second centrifugation as cytosol. It was divided into aliquots and stored at -60°C until used.

[3H]PDBU binding was determined in 250 μl of incubation mixtures, containing [3H]PDBU, mouse brain cytosol (15 to 50 μg), 0.05 mM Tris-Cl, pH 7.4, 0.1 mM CaCl_2, phosphatidylserine at 100 μg/ml, nonradioactive phorbol esters where indicated, and bovine γ-globulin in 2 to 4 mg/ml. In competition experiments, the concentration of free [3H]PDBU at the 50% effective dose was 3.5 to 5.7 nM. For determination of PDBU saturation curves, concentrations of free [3H]PDBU between 0.1 and 40 nM were used. Dilutions of [3H]PDBU and, when added to the aqueous phase, nonradioactive phorbol esters were made in 0.05 mM Tris-Cl, pH 7.4, containing bovine γ-globulin at a concentration of 10 mg/ml. In competition experiments in which nonradioactive phorbol esters were added to the lipid phase of the assay, dilutions of the phorbol ester in chloroform or in methanol were mixed with the phosphatidylserine dissolved in chloroform, after which the solvent was evaporated under a stream of N_2. Mixed liposomes were then prepared by addition of 0.05 mM Tris-Cl, 7.4, and sonication for one 30-sec burst with the microtip probe of a Sonifier Cell Disrupter (Heat Systems-Ultrasonics, Plainview, NY).

Incubations were carried out in 1.5 ml polystyrene Eppendorf microcentrifuge tubes for 30 min at 37°C. The samples were then chilled for 5 min at 0°C, 187 μl of 35% polyethylene glycol in 50 mM Tris-Cl pH 7.4, (w:w) was added (to bring the final polyethylene glycol concentration to 15%), and samples were incubated for 15 min at 0°C to permit precipitation of the proteins. The precipitates were spun down at 12,000 rpm in a Beckman microfuge 12 centrifuge at 4°C. A 100-μl aliquot of the supernatant was removed, and its radioactivity was measured to determine the free [3H]PDBU concentration. The remainder of the supernatant was removed by aspiration and blotting with a Kimwipe, the tip of the centrifuge was cut off, and the radioactivity in the pellet was measured to determine total bound [3H]PDBU. Nonspecific binding was measured in the presence of 30 μM nonradioactive PDBU, and the partition coefficient for [3H]PDBU between supernatant and pellet was determined. Specific binding represents the difference between the total and nonspecific binding, where nonspecific binding for each tube was calculated from the partition coefficient of [3H]PDBU and the measured free [3H]-PDBU concentration for that tube. In each experiment, each point represents the average of triplicate or quadruplicate determinations. Each experiment was performed at least twice (see text for actual number). In competition experiments, ID_50 values were determined by a least-squares fit of the data, assuming a theoretical competition curve. The K_i was calculated from the ID_50 according to the relationship:

\[ K_i = \frac{I_D}{1 + L/K_d} \]

where L is concentration of free [3H]PDBU at the ID_50, and K_i is dissociation constant for [3H]PDBU under these assay conditions.

Inclusion of carrier protein is necessary in the binding assay to stabilize the free [3H]PDBU (17) as well as to allow efficient precipitation. Using brain membranes, we had previously shown that the presence or absence of bovine serum albumin at 4 mg/ml did not affect the measured [3H]-PDBU affinity (17). Because bovine serum albumin has a fatty acid binding site which might interact in lipid reconstitution studies, we now routinely use bovine γ-globulin instead. We have confirmed that [3H]PDBU binding affinity is essentially independent of the amount of bovine γ-globulin included in the assay over the range of 0.4 to 4.0 mg/ml.

RESULTS

The identification of the cytosolic aporeceptor has made it possible to manipulate the lipid portion of the receptor separately from the protein portion. It is therefore now feasible to examine the effects of highly lipophilic compounds on the receptor by incorporating the compounds directly into the phospholipid liposomes used to reconstitute the aporeceptor. Using such methods, we were able to demonstrate that the diacylglycerol derivative diolen competitively inhibited phorbol ester binding (39) and that the diolen was unable to transfer in aqueous solution between liposomes (7).

We have now examined the apparent binding potencies of a series of symmetrically substituted phorbol 12,13-diesters with fatty acid side chains varying in length from 10 to 18 carbon atoms. This series of derivatives ranges from one of good aqueous solubility to another that is almost totally oil soluble. The identification of these compounds and the rationale for their selection are described elsewhere (27).

Table 1 Apparent K_i values for lipophilic phorbol esters as a function of their manner of addition

<table>
<thead>
<tr>
<th>Ligand</th>
<th>lipid phase</th>
<th>Aqueous phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDD</td>
<td>2.4 ± 0.2 (n = 2)</td>
<td>3.2 ± 0.8 (n = 2)</td>
</tr>
<tr>
<td>PDD</td>
<td>17 ± 9 (n = 4)</td>
<td>560 ± 130 (n = 3)</td>
</tr>
<tr>
<td>PDD</td>
<td>34 ± 11 (n = 4)</td>
<td>8,000 ± 1,200 (n = 3)</td>
</tr>
<tr>
<td>PDD</td>
<td>9.0 ± 1.5 (n = 2)</td>
<td>4,200 ± 400 (n = 3)</td>
</tr>
<tr>
<td>PDD</td>
<td>7.4 ± 0.8 (n = 4)</td>
<td>24,000 ± 1,000 (n = 2)</td>
</tr>
</tbody>
</table>

*Data are from Thielmann and Hecker (41). *ID_50: dose inducing ear reddening in one-half of a group of mice (see Ref. 26 for a description of the assay; units are mmol/ear). NT, not tested.

<table>
<thead>
<tr>
<th>Site of addition</th>
<th>Biological response of mouse ear reddening (ID_50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDD</td>
<td>0.01</td>
</tr>
<tr>
<td>PDD</td>
<td>0.29</td>
</tr>
<tr>
<td>PDD</td>
<td>14.0</td>
</tr>
<tr>
<td>PDD</td>
<td>&gt;60</td>
</tr>
<tr>
<td>PDD</td>
<td>NT</td>
</tr>
<tr>
<td>PDD</td>
<td>NT</td>
</tr>
<tr>
<td>PDD</td>
<td>NT</td>
</tr>
</tbody>
</table>

*Data are from Thielmann and Hecker (41). Mean ± S. E. (n > 2) or range (n = 2) for n experiments. *Extrapolated value estimated graphically. Maximum inhibition at 100 μM was 36%.

CANCER RESEARCH VOL. 45 JANUARY 1985

20
LIPOPHILIC PHORBOL ESTERS

Chart 1. Inhibition of \(^{3}H\)PDBU binding by lipophilic phorbol esters as a function of their mode of addition and the presence of detergent. The ability of PDS (A) or PDD (B) to compete for \(^{3}H\)PDBU binding to the cytosolic aporeceptor was assayed under 3 different conditions. PDS and PDD either were incorporated directly into the phosphatidylycerine used to reconstitute the aporeceptor or else were added independently in aqueous suspension (C, Δ). The mixture of phorbol ester and phospholipids was then either used immediately in the aporeceptor assay (C, Δ) or else was first preincubated overnight at 37° with 0.0375% Triton X-100 before addition to the assay (Δ) (see "Materials and Methods" for details). Points are the average of triplicate determinations in single experiments. Each experiment was performed 2 to 4 times, and the results illustrated are representative. Apparent Kᵦ values for PDS added to the lipid and aqueous phases and incubated with Triton X-100 were 4.1 ± 1.5 nM (mean ± S.E., n = 4) and 13.6 ± 0.4 nM (mean ± range, n = 2), respectively. The apparent Kᵦ value for PDD added to the lipid phase and incubated with Triton X-100 was 5.0 ± 1.2 nM (mean ± range; n = 2). Kᵦ values were calculated using the Kᵦ values for \(^{3}H\)PDBU under the corresponding assay conditions. As determined by Scatchard analyses, these were 0.96 ± 0.09 nM (n = 5) and 4.18 ± 0.03 nM (n = 2) for assays without and with overnight incubation with Triton X-100, respectively. Note that this difference in Kᵦ will lead to different values of the expression 1 + L/Kᵦ used to derive the Kᵦ from the ID₅₀. Bars, S.E.

solubilities of 2 μM and 50 nM for PMA and PDD, respectively (28), these latter derivatives would also be expected to show very low aqueous solubilities.

When the phorbol esters were mixed in organic solvents with phosphatidylycerine and mixed liposomes prepared, all of the derivatives inhibited \(^{3}H\)PDBU binding with high apparent affinities (Table 1). Apparent Kᵦ values fell between 2.4 nM for PDD and 34 nM for PDM. These values contrast with the marked differences in biological potencies reported in the literature (Table 1). A representative competition curve for PDS is illustrated in Chart 1. One for PDL is shown in Chart 3. The shapes of the curves drawn are those theoretically expected. As can be seen, the actual data points delineate slightly shallower curves than theoretically expected. Although we do not know the reason, the deviations from the theoretical may reflect heterogeneity in the state of association of the lipophilic phorbol esters with the phospholipid.

The usual protocol for in vitro assays or binding analysis involves the addition to an aqueous mixture of a concentrated solution of the phorbol ester dissolved in an organic solvent such as dimethyl sulfoxide or methanol. Assayed under such conditions, the derivatives varied markedly in apparent binding affinities (Table 1). PDD was highly active. Its apparent Kᵦ, 3.2 nM, was similar to that determined for the ligand incorporated into the liposomes. PDL showed substantial loss of activity, and the derivatives PDM, PDS, and PDO yielded apparent Kᵦ values of >4000 nM.

The probable explanation for the decreased activity under these conditions of the more lipophilic derivatives is their inability to efficiently transfer through the aqueous solution into the liposomes. Since detergent should facilitate the transfer process, we examined the effect of a low concentration of Triton X-100 on the apparent activity of PDS added to the aqueous phase. Overnight preincubation in the presence of 0.0375% Triton X-100 led to a dramatic restoration in apparent binding affinity, to 13.6 ± 0.4 nM (n = 2 experiments) (Chart 1A). In contrast, only
LIPOPHILIC PHORBOL ESTERS

Chart 2. Inhibition of [3H]PDBU binding by PDS as a function of phospholipid concentration. The Kᵦ for inhibition of [3H]PDBU binding by PDS incorporated into phosphatidyserine liposomes was determined at the indicated phosphatidyserine concentrations. Each point represents the Kᵦ value derived from a single competition curve: ---, the expected relationships if the Kᵦ was independent of the phosphatidyserine concentration; ——, the expected relationships if the Kᵦ was directly proportional to the phosphatidyserine concentration.

...a small shift in apparent PDS binding affinity resulted in the control experiments with PDS incorporated directly into the phosphatidyserine liposomes. The apparent Kᵦ, 4.1 ± 1.5 nM (n = 4 experiments), was within 2.5-fold of that measured in the absence of Triton X-100.

Unlike PDS, the more hydrophilic PDD had shown similar activity independent of its manner of addition. As would thus be predicted, overnight incubation in the presence of Triton X-100 of the phosphatidyserine liposomes with PDD added to the aqueous phase had very little effect on binding affinity (Chart 1B).

The apparent affinity for a highly lipophilic ligand present in mixed liposomes should reflect its local concentration in the liposomes rather than the degree of dispersion of the liposomes by the aqueous phase. The apparent Kᵦ, expressed in molar units, should therefore decrease (i.e., apparent affinity should increase) as the liposomes are diluted. We had previously shown that diolein behaved in such a fashion (39). We have now found that PDS behaves similarly (Chart 2). It should be noted that the fit to the theoretical curve is not precise, however, perhaps due to loss at the air-water interface.

To better compare the relative binding affinities of phorbol esters and diacylglycerols, we determined the inhibitory activity of dilaurin (1,2-glycerol dilaurate) incorporated into phosphatidyserine liposomes and assayed under the same conditions used to determine the activity of PDO. An apparent Kᵦ of 290 ± 30 nM (S.E.; ± range, n = 2; 0.13%, w:w, relative to phosphatidyserine) was obtained, indicating a 17-fold difference in affinities (Chart 3). Comparison of our previously published Kᵦ for diolein (0.35%, assayed under somewhat different conditions) (39) with that for PDO indicated a 76-fold difference. These differences in affinities for homologous pairs of compounds are thus considerably less than observed earlier for the heterologous pair of PDBU and diolein.

DISCUSSION

Previous evidence for the analogy between the phorbol esters and diacylglycerols includes their stimulation of protein kinase C in a similar fashion (10), their induction of certain similar, rapid responses in cells (29, 36, 40), and the competitive inhibition of [3H]PDBU binding by both classes of agents (39). The evidence presented here that the membrane dissolved form of the phorbol esters is recognized as is that of diolein further strengthens the analogy. Additional support comes from the comparison of phorbol and glycerol derivatives with the same side chains, which indicates less difference in affinities than had been suggested initially from comparison of PDBU and diolein.

Despite the similarities, it should be noted that the 2 classes of compounds show a somewhat different dependence on side chain structure. Unlike diolein, distearin failed to inhibit [3H]PDBU binding (39). In contrast, PDS is similar in binding potency to PDO.

Protein kinase C is found in both the cytoplasmic and membrane fractions, as defined operationally after lysis of cells in the presence of chelating agents. Kraft et al. (31, 32) have reported that exposure of cells to the phorbol esters induces the rapid shift of protein kinase C from the cytoplasmic to the membrane fraction. This shift could result from initial interaction of the...
The evidence presented here that the receptor recognizes the translocation of the complex to the membranes. Alternatively, it might reflect stabilization by phorbol ester dissolved in the membranes of the membrane associated state of protein kinase C. The evidence presented here that the receptor recognizes the membrane dissolved form of the phorbol esters, together with the findings by us and others that the aporeceptor is dependent on phospholipids for phorbol ester binding, strongly favors the latter model.

The apparent binding activities of the lipophilic phorbol esters PDD, PDL, and PDM, when added to the aqueous phase of the binding assay mixture, show general agreement with the biological potencies of these compounds, as determined in the mouse ear reddening assay (Table 1). Our earlier analysis of the activities of PDD and PDM for induction of fibronectin loss in chicken embryo fibroblasts had likewise correlated with the results of the ear reddening assay (16). Since the variation in apparent binding affinities of these derivatives in vitro appears to reflect inefficient transfer to the receptor rather than intrinsic potency, it seems likely that such pharmacokinetic factors predominate in the biological systems as well.

Extrapolation of the decreasing activities of PDD, PDL, and PDM when added to the aqueous phase would have suggested even lower activities for PDO and PDS than were observed. The existence of more than one mechanism of transfer of phorbol ester to the liposomes, e.g., both transfer of phorbol ester monomers dissolved in aqueous solution and also fusion with phorbol ester micelles, might account for this discrepancy. Alternatively, the results could be explained by as little as 0.01% hydrolysis, whether enzymatic or spontaneous, of the phorbol diester to the corresponding, biologically potent phorbol 13-monooesters.

The lack of activity of phorbol, as contrasted to its esters, both in vivo (27) and in vitro (15), has strongly indicated that adequate hydrophobicity was one of the requirements for activity. In addition, the thermodynamics of PDBU binding to the receptor in mouse brain membranes had indicated that the binding was entropy driven, consistent with a hydrophobic interaction (18). A priori, this hydrophobic interaction could be with either protein, lipid, or a combination of both. Our demonstration that a photoactivatable phorbol ester, when bound to the receptor, specifically labeled phosphatidylserine and phosphatidylethanolamine (11) indicated that the bound phorbol ester was in close proximity to phospholipid. The present results argue that the phorbol ester may first interact with the phospholipid before being recognized by the phospholipid-protein receptor complex.

Physical-chemical studies have begun to characterize the interactions between the phorbol esters and phospholipids (13, 28). These various observations predict, and we have indeed confirmed (7), that different lipid environments should be able to generate different binding affinities. Since long-term effects of phorbol ester treatment include changes in phospholipid metabolism (22, 23) and in membrane fluidity (9, 20), such changes may provide a mechanism for modifying phorbol ester binding and response.

Conceptually, it is important to distinguish interactions of phorbol esters with phospholipids which affect binding behavior with the aporeceptor from specific, high-affinity binding of phorbol esters to lipids alone. Two studies had suggested that the latter might occur. Esumi and Fujiki (19) reported saturable binding of PMA to sphingomyelin vesicles. However, 50% inhibition of binding only occurred with PMA at \( \mu \text{M} \) concentrations and at a 1:2 molar ratio of PMA to phospholipid. In contrast, we and others find that the cellular phorbol ester receptor binds PMA at \( \mu \text{M} \) concentrations or below (6). In the second study, Deleers and Malaise (12) reported specific binding of PDBU to phosphatidylinoline liposomes. Problems with this study include both the low proportion of specific binding, 5 to 21%, and the structure-activity relationships. 4\( \alpha \)-PDD was only 7-fold less potent than PDD at inhibiting binding, whereas it is entirely inactive biologically at the cellular receptor (6).

Although partitioning of the phorbol ester into the lipid bilayer may be an important factor in determining activity, the relationship is more complex, as evidenced by the variation in phorbol diester affinities when values are expressed in terms of membrane concentrations. For example, the \( K_a \) of PDBU under our assay conditions is 0.2 pmol/mg of phosphatidylserine, based on the measured partition coefficient. In contrast, the \( K_a \) for PDS, assuming it is present entirely in the phosphatidylserine, is 90 pmol/mg of phosphatidylserine.

As summarized elsewhere, a number of studies have reported evidence for subclasses of phorbol ester responses distinguishable on the basis of structure-activity relations (6). Although such findings have typically been interpreted in terms of differences in equilibrium binding affinities, the present study suggests that differences in kinetics of equilibration might provide an alternative possible mechanism. The location at which protein kinase C is activated within the cell would be expected to determine the substrates to which it has access. This distribution of activated protein kinase C should reflect, among other factors, the distribution of the phorbol ester within the different membranes of the cell. A phorbol ester which only slowly translocated from the plasma membrane to the nuclear membrane might therefore induce a generally similar but not identical spectrum of responses compared to a phorbol ester which equilibrated more rapidly. The src tyrosine kinase may provide an example of this type of regulation. Mutations affecting its location although not its enzymatic activity also alter its spectrum of biological effects (33).

ACKNOWLEDGMENTS

We thank Dr. S. H. Yuspa for his careful reading of the manuscript and Maxine Bellman for her expert typing.

REFERENCES

LIPOPHILIC PHORBOL ESTERS

184.


Highly Lipophilic Phorbol Esters as Inhibitors of Specific $[^3]$HPhorbol 12,13-Dibutryrate Binding

Nancy A. Sharkey and Peter M. Blumberg