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Research Article

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Receptor-mediated Modulation of Human Monocyte, Neutrophil, Lymphocyte, and Platelet Function by Phorbol Diesters

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ABSTRACT The tumor promoting phorbol diesters elicit a variety of responses from normal and leukemic blood cells *in vitro* by apparently interacting with cellular receptors. The biologically active ligand [20-³H] phorbol 12,13-dibutyrate ([³H]PDBu) bound specifically to intact human lymphocytes, monocytes, polymorphonuclear leukocytes (PMN), and platelets, but not to erythrocytes. Binding, which was comparable for all four blood cell types, occurred rapidly at 23° and 37°C, reaching a maximum by 20–30 min usually followed by a 30–40% decrease in cell associated radioactivity over the next 30–60 min. The time course for binding was temperature dependent with equilibrium binding occurring after 120–150 min at 4°C, with no subsequent loss of cell-associated radioactivity at this temperature. Bound [³H]PDBu could be eluted by addition of unlabeled PDBu. Scatchard analysis of data from 4°C binding studies revealed linear plots with high affinity receptors in these cell types with dissociation constants and receptors per cell of 60 nM and 7.8×10^5 /cell for lymphocytes, 51 nM and 15.5×10^5 /cell for monocytes, 38 nM and 4.0×10^5 /cell for PMN, and 19 nM and 2.9×10^4 /cell for platelets. Structure-activity studies using unlabeled phorbol-related compounds demonstrated a close correlation between their abilities to inhibit binding of [³H]PDBu to cells and their abilities to induce cellular responses (monocyte and PMN H₂O₂ secretion, lymphocyte ³HTdR incorporation, and platelet tritiated serotonin release); phorbol and 4- α phorbol were inactive while phorbol 12-myristate 13-acetate (PMA), PDBu, mezerein, and phorbol 12,13-diacetate (in de-

creasing order of potency) inhibited [³H]PDBu binding and elicited the various responses. Thus, these high affinity, specific receptors for the phorbol diesters, present on monocytes, lymphocytes, PMN, and platelets, mediate the pleiotypic effects induced by these ligands.

INTRODUCTION

Tumor promoting agents are substances, which although noncarcinogenic themselves, cause tumor formation when applied repeatedly to mouse skin that has been previously treated with a subthreshold dose of a carcinogen. The most potent promoters in the mouse skin system are esters of the tetracyclic diterpene phorbol (1, 2). The phorbol esters, *in vitro*, cause a variety of biological responses in many different cell types, including normal and leukemic blood cells (3–6). These include stimulation of hydrogen peroxide (H₂O₂) and superoxide anion (O₂⁻) production by polymorphonuclear leukocytes (PMN)¹ and monocytes (7–9), aggregation and serotonin release by platelets (10, 11), and mitogenesis in lymphocytes (12, 13).

The phorbol esters bind in a specific, saturable, reversible fashion to a variety of cells and cell particulate fractions (14–17). Their binding affinities generally are comparable to their abilities to induce cellular changes *in vitro*, such as the loss of surface fibronectin from

¹ *Abbreviations used in this paper:* DMM, Dulbecco's Modified Eagle Medium; [³H]5HT, tritiated hydroxytryptamine binoxalate 5-[1,2-³H(N)]-serotonin; [³H]PDBu, 20-³H phorbol 12,13-dibutyrate; MZ, mezerein; PDA, phorbol 12,13 diacetate; PHR, phorbol; 4 α -PHR, 4 α -phorbol; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear leukocyte; PRP, platelet-rich plasma.

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chicken embryo fibroblasts (14) and the production of interleukin 2 by lymphocytes (17). Likewise, the binding affinities of the phorbol esters seem to parallel their tumor-promoting potencies in the mouse skin model (14–18). It thus appears that these substances initiate their effects by binding to a specific cellular receptor.

In this study, using [20-³H] phorbol 12,13-dibutyrate ([³H]PDBu), we demonstrate and characterize the specific binding of this labeled phorbol ester to human PMN, monocytes, lymphocytes, and platelets. In addition, we show that the binding affinities (K_d) of [³H]PDBu and the inhibitory constants (K_i) of related unlabeled compounds, correlate well with the doses required to elicit 50% of the maximal level of selected responses by these blood cells (ED50).

METHODS

Dulbecco's modified Eagle medium (DMM, Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) was formulated with endotoxin-free water and supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 20 mM Hepes, 1 mg/ml dextrose, and sterilized by filtration. Ficoll-sodium diatrizoate, specific gravity 1.077 (Histopaque 1077), dextran (mol wt = 500,000), horseradish peroxidase (type II), and superoxide dismutase were obtained from Sigma Chemical Co., St. Louis, MO. Gey's balanced salt solution with 2% bovalbumin (Gey's-BSA) was from Flow Laboratories, Inc., Rockville, MD. Polyvinyl pyrrolidone-coated silica (Percoll) and Sepharose 2B were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. [³H]PDBu, as well as tritiated hydroxytryptamine binoxalate (5-[1,2-³H(N)]-serotonin) ([³H]5HT) and thymidine [*methyl*-³H]thymidine) were purchased from New England Nuclear, Boston, MA. Phorbol (PHR), phorbol 12-myristate 13-acetate (PMA), phorbol 12,13 diacetate (PDA), 4 α -phorbol (4 α -PHR), and mezerein (MZ) were from P-L Biochemicals, Inc., Milwaukee, WI.

Isolation of blood cells. Peripheral blood was obtained from normal donors after their informed consent according to a protocol approved by the Duke University Clinical Investigation Committee. Monocytes and lymphocytes were isolated from heparinized blood (10 U/ml), as described (19), using sequential Ficoll-sodium diatrizoate separation and discontinuous Percoll gradient centrifugation. Following the wash steps, the cells from the monocyte and lymphocyte bands were suspended in DMM for use in experiments. Based on Wright's and nonspecific esterase staining, the monocytes and lymphocytes were >85% pure. PMN were separated from heparinized blood by a described method (20) using Ficoll-sodium diatrizoate sedimentation for removal of mononuclear cells followed by dextran (3%) sedimentation of the remaining erythrocytes and buffy coat. There was <1% contamination by mononuclear cells and hypotonic lysis removed remaining erythrocytes. PMN were suspended in DMM for most experiments. Platelets were obtained using a modification of the gel filtration method of Tangen (21). Column buffer consisted of 0.137 M NaCl, 0.0056 M dextrose, 0.0027 M KCl, 0.00042 M Na H₂PO₄, 0.012 M NaHCO₃, and 0.00011 M EDTA. Before experiments, 1 part 0.025 M CaCl₂ was added to 9 parts buffer and platelets. Erythrocytes were obtained after Ficoll-sodium diatrizoate sedimentation of heparinized blood from the cells in the lowermost portion of the tubes. They were washed and found to be leukocyte free and suspended to DMM for experiments.

Binding assays. The standard binding assay was done in 5 ml polypropylene tubes (Walter Sarstedt, Inc., Princeton, NJ) containing a final volume of 0.2 ml. 5–10 × 10⁵ blood cells (or 1 × 10⁷ for platelet experiments) in 0.1 ml of DMM for PMN, lymphocyte, monocyte, and erythrocyte assays, or 0.1 ml of platelet buffer for platelet assays were added to each tube, and 0.1 ml Gey's-BSA containing the experimental concentration of [³H]PDBu or [³H]PDBu plus potential inhibitors of binding was added. To determine nonspecific binding, all experiments were done in the presence and absence of 30 µM unlabeled PDBu. All results are presented as specific binding (that is, the difference between [³H]PDBu bound in the presence and absence of this excess unlabeled PDBu). Binding assays were carried out in triplicate and additives were allowed to reach the experimental temperature [4°, 23°, or 37°C] before being placed together. Following incubation at a specific temperature for a desired length of time, 4 ml of cold assay wash buffer (19) was added to each tube and the cells and solution aspirated from the tubes with a semiautomated sample harvester (Otto Hiller Co., Madison, WI) using glass fiber filter paper (Whatman 5679, grade 943AH, Whatman Inc., Paper Div., Clifton, NJ). Each tube was then washed with an additional 4 ml of cold assay wash buffer. The filters were counted in Aquasol-2 (New England Nuclear) using a Packard Tricarb Scintillation Counter (3375, Packard Instrument Co., Inc., Downers Grove, IL).

Platelet serotonin release. Platelet serotonin release was measured with a modification of a described technique (22). Platelet-rich plasma (PRP) was incubated with [³H]5HT (1 µCi/ml PRP) at 37°C for 30 min before gel filtration over Sepharose 2B. 0.45 ml of gel-filtered, [³H]5HT-labeled platelets (1–2 × 10⁸ platelets/ml) in platelet column buffer were incubated with 0.05 ml of the potential stimulator of serotonin release in platelet column buffer (10 × final experimental concentration) or 0.05 ml platelet column buffer (for a blank) at 37°C for 15 min. 0.05 ml of 50 mM cold EDTA was added, and the samples were centrifuged for 5 min in a Beckman microfuge (Beckman Instruments, Inc., Fullerton, CA). 0.2 ml of supernatant was removed and radioactivity counted in Aquasol-2. 0.2 ml of a lysate consisting of 0.45 ml [³H]5HT serotonin-labeled platelets, 0.05 ml 50 mM EDTA, and 0.05 ml 20% Triton X-100 was also counted. Percent release was calculated using the formula: [(counts per minute in sample supernatant-counts per minute in blank) ÷ (counts per minute in lysate-counts per minute in blank)] × 100.

Hydrogen peroxide production. This assay was done in 96 chamber microtitre plates (Microtest II, No. 3040, Falcon Labware, Div. Becton-Dickinson & Co., Oxnard, CA) using a modification of a described method (23). 1–2 × 10⁵ monocytes or PMN were incubated in a total volume of 0.2 ml assay buffer containing 1 mg/ml glucose, 19 U/ml horseradish peroxidase, and 2 g/liter phenol red plus varying concentrations of potential stimulants for H₂O₂ release for 1 h at 37°C. The plates were then read using a Multiskan automatic eight-channel photometer (Flow Laboratories, Inc.) at 600 nm after alkalization with 0.01 ml 1 N NaOH. H₂O₂ production was calculated by construction of a standard curve with exogenous H₂O₂.

Thymidine incorporation. Lymphocytes (3–5 × 10⁵/well) were cultured in DMM with or without serum in the presence of various concentrations of potential stimulants of thymidine incorporation for 24 h in microtitre plates. 1 µCi of tritiated thymidine was added per microtitre chamber and the cells allowed to incubate at 37°C for an additional 24 h. At the end of this time, the cells were harvested as described

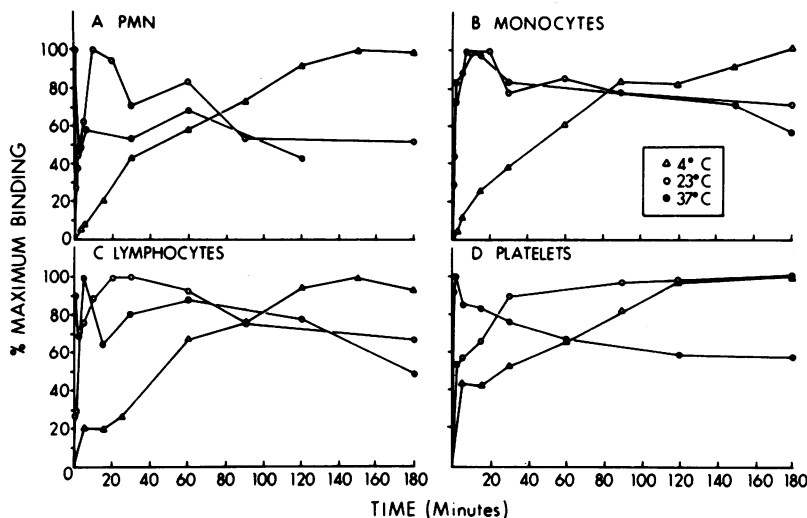


FIGURE 1 Representative time courses of specific [^3H]PDBu binding to intact human PMN (A), monocytes (B), lymphocytes (C), and platelets (D) at 4°C (Δ), 23°C (\circ), and 37°C (\bullet). Specific binding was measured using 50 nM [^3H]PDBu and 30 μM unlabeled PDBu. Each point is the mean of three replicates and results are expressed as percentage of maximum binding occurring at that temperature. SEM for the triplicates ranged from 0.5 to 5.8%. Similar results to those displayed in this figure were seen in two other separate experiments using PMN, monocytes, and lymphocytes and in one other using platelets.

for binding assays and cell associated radioactivity counted in Aquasol-2.

RESULTS

General characteristics of [^3H]PDBu binding. There was specific binding of [^3H]PDBu to PMN, lymphocytes, monocytes, and platelets, but as previously reported (15), erythrocytes did not specifically bind the [^3H]PDBu. Nonspecific binding of the ligand was generally linear with respect to the concentration of [^3H]PDBu added and at maximum, accounted for <30% of the total [^3H]PDBu bound or <1% of the [^3H]PDBu added. The time course for the specific binding of [^3H]PDBu varied with temperature (4°, 23°, and 37°C) but was generally similar for PMN, lymphocytes, monocytes, and platelets, at a given temperature (representative time courses are seen in Fig. 1). Binding was rapid at 23° and 37°C, reaching a maximum by 1–5 min at 37°C and by 20–30 min at 23°C. This was followed by a 30–40% decline in cell-associated radioactivity over the next 2–3 h in all instances except for platelets at 23°C. While seen in all experiments, the rate of this decline in cell-associated radioactivity was somewhat variable. Dissociated radioactivity was found in the media, and analysis by thin-layer chromatography after 3 h showed no degradation of the [^3H]PDBu.² At 4°C there was a slower increase in spe-

cific binding of [^3H]PDBu with time, reaching a maximum by 120–150 min. As opposed to experiments done at 23° or 37°C, where a stable plateau of binding was not achieved, no subsequent loss of bound material was noted after maximum binding had been reached at 4°C.

Comparable to the rate of association of the ligand, the rate of dissociation of cell-associated radioactivity, following the addition of excess unlabeled PDBu, was rapid at 23°C, with <10% of the initial radioactivity remaining after 15 min (Fig. 2). The dissociation was much slower at 4°C, paralleling the slower rate of binding at this temperature.

To further characterize [^3H]PDBu binding as a function of ligand concentration, binding assays were done at 4°C for 2–3 h. Mean data for specific binding to lymphocytes, monocytes, PMN, and platelets are presented in Fig. 3. Scatchard analysis of the binding data revealed linear curves for all of these blood cells suggesting the presence of one class of binding site in each case (Fig. 4). The K_d were similar for all four cell types, however, the calculated number of binding sites per cell was more variable. With the lack of development of steady-state binding in the time course experiments at 23°C and 37°C, it was felt that valid Scatchard analysis of [^3H]PDBu binding could be made only at 4°C. Mean values for K_d and number of sites per cell for the blood cells at this temperature are given in Table I.

Specificity of binding. The relative potency of the

² Weinberg, J. B., and B. J. Goodwin. Manuscript in preparation.

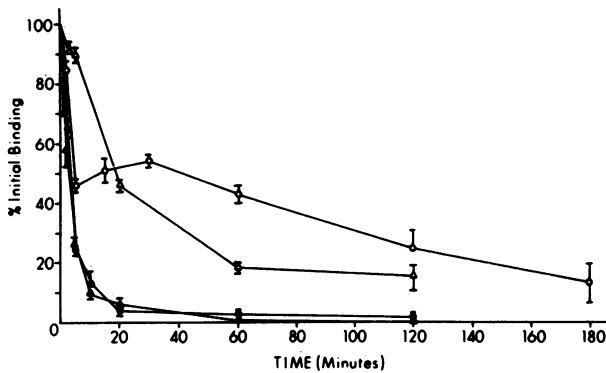


FIGURE 2 Dissociation of specifically bound [³H]PDBu from monocytes (●) and lymphocytes (△) at 4° and 23°C following the addition of excess (20 μM) unlabeled PDBu. Binding was done for 30 min at 23°C and for 60 min at 4°C using 50 nM [³H]PDBu in the absence and presence of 30 μM unlabeled PDBu before the addition of the excess unlabeled PDBu at time 0. The points and bars represent the mean of three replicates ±1 SEM.

phorbol esters and related compounds to promote tumor formation in vivo and to cause cellular changes in vitro is highly dependent on their structure (24). In other cell types, the K_i for [³H]PDBu binding of various phorbol derivatives correlate well with their potency in evoking these in vivo and in vitro changes. The inhibition of [³H]PDBu binding to lymphocytes, monocytes, and PMN by various phorbol esters and MZ was examined. In these cell types, binding was inhibited by PDA, PMA, PDBu, and MZ but not by the PHR (Fig. 5). The order of potency of these compounds for inhibition of [³H]PDBu binding paralleled their biological potency as tumor promoters in the mouse skin model (25). That is, PMA was the most potent inhibitor of binding followed by PDBu

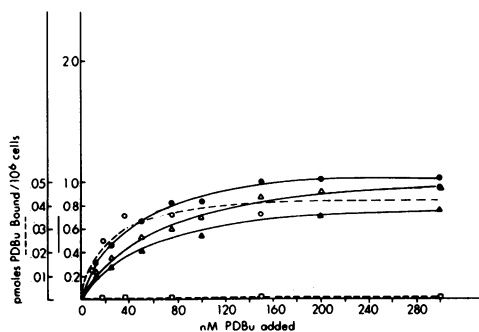


FIGURE 3 Representative equilibrium specific [³H]PDBu binding to intact human monocytes (●); lymphocytes (△), PMN (▲), platelets (○), and erythrocytes (□) as a function of ligand concentration. Incubation was at 4°C in the absence and presence of 30 μM unlabeled PDBu for 2 h. Each point represents the mean of triplicates and the standard errors ranged from 1.2 to 6.6% of the mean.

and then MZ and PDA, while PHR, biologically inactive as a promoter, was not inhibitory of [³H]PDBu binding. The apparent K_i for this inhibition are shown in Table II.

Correlation of [³H]PDBu binding with cellular functions. Lymphocytes cultured with increasing concentrations of PMA showed a dose-dependent rise in tritiated thymidine incorporation with an ED₅₀ of 6 nM (Fig. 6A). There was a similar dose-dependent increase in stimulation index with increasing PDBu concentrations but the ED₅₀(40 nM) was higher than that for PMA. This ED₅₀ value for PDBu stimulation is similar to the K_d of 60 nM for [³H]PDBu binding to lymphocytes. Unlike the biologically active phorbol esters, the nonpromoting substance 4α-PHR caused no increase in stimulation index with escalating concentrations. At the higher concentrations of PMA and PDBu, in the absence of serum, the stimulation indices diminished, suggesting toxicity to the cells. Experiments done in the presence of 10% fetal bovine serum showed greater overall stimulation of thymidine incorporation and substantially eliminated this apparent toxic effect of the higher phorbol ester concentrations (Fig. 6B). This effect of serum, in addition to its more general beneficial effect on cultured cell growth, could be related to the binding of PMA and PDBu to a protein in the serum, recently characterized by Shoyab and Todaro (27), which binds [³H]PDBu and competitively inhibits the binding of [³H]PDBu to its specific cellular receptors. ED₅₀ were higher than those in the absence of serum (420 nM for PMA and 1,000 nM for PDBu). Once again, 4α-PHR caused no increase in stimulation index.

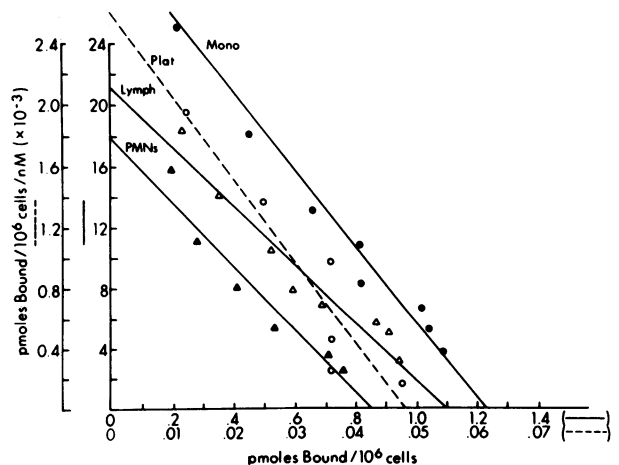


FIGURE 4 Scatchard plot of equilibrium [³H]PDBu binding data presented in Fig. 2 for human monocytes (●); lymphocytes (△), PMN (▲), and platelets (○). The lines were determined by linear regression with $r = 0.98$ (monocytes), $r = 0.98$ (lymphocytes), $r = 0.98$ (PMN), and $r = 0.96$ (platelets).

TABLE I
Mean K_d and Sites/Cell for [3 H]PDBu Binding to Normal Human Blood Cells at 4°C

Cell type	K_d	Mean number sites per cell
Monocytes	51±6 nM (n = 5)	15.5 × 10 ⁵ ±4.3 × 10 ⁵
Lymphocytes	60±4 nM (n = 4)	7.8 × 10 ⁵ ±2.7 × 10 ⁵
PMN	38±9 nM (n = 2)	4.0 × 10 ⁵ ±1.1 × 10 ⁵
Platelets	19 nM (n = 1)	2.9 × 10 ⁴
Erythrocytes	° (n = 2)	°

° No detectable binding.

TABLE II
 K_i for Inhibition of [3 H]PDBu Binding of Phorbol-related Compounds

Inhibiting substance	Cell type		
	PMN	Lymphocytes	Monocytes
PMA	75 nM	18 nM	65 nM
PDBu	100 nM	60 nM	165 nM
MZ	1,000 nM	200 nM	350 nM
PDA	30 μM	4 μM	5 μM
PHR	°	°	°

° No inhibition noted.

PDBu stimulated the production of H₂O₂ by human monocytes and PMN in a dose-dependent fashion with an ED₅₀ of 42 nM for both these cell types. This ED₅₀ correlated well with the K_d of [3 H]PDBu binding to monocytes (51 nM) and PMN (38 nM). PMA, PDA, and mezerein likewise caused increased H₂O₂ production by monocytes and PMN (Fig. 7) with ED₅₀ as displayed in Table III, while the biologically inactive compounds 4α-PHR and PHR caused no increased release. Although MZ caused a higher maximum H₂O₂ secretion by monocytes in Fig. 7, this was not the case in other comparable experiments and in experiments using mouse peritoneal macrophages (data not shown).

PDBu stimulated the release of [3 H]5HT from platelets in a dose-dependent fashion (Fig. 8). The ED₅₀ of 18 nM correlated closely with the K_d for [3 H]PDBu binding to platelets of 19 nM. Likewise, PMA, the more potent tumor-promoting substance, caused a dose-dependent increase in [3 H]5HT release, but with a lower ED₅₀ of

3 nM. PHR and 4 α-PHR caused no increase in [3 H]5HT release.

DISCUSSION

Recent studies support the hypothesis that the primary interaction of the phorbol esters is with specific binding sites on the cell surface, and that they may mediate at least some of their pleiotypic cellular effects via these receptors (14–18). In the present report, using [3 H]PDBu as a ligand, we have provided evidence that normal human blood lymphocytes, monocytes, PMN, and platelets have specific receptors for [3 H]PDBu and the related phorbol esters, while erythrocytes do not. In addition, we demonstrated the close correlation between the affinities of the phorbol esters for these receptors and their ability to evoke the biological responses of H₂O₂ generation by PMN and monocytes,

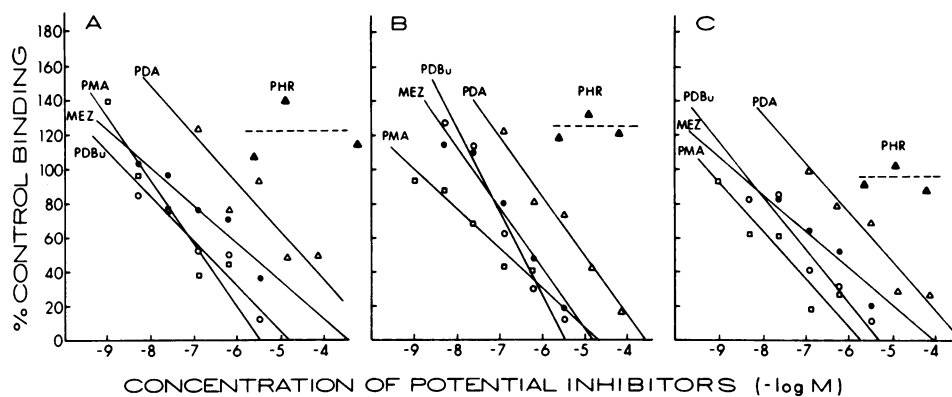


FIGURE 5 Dose-response curves for inhibition of specific [3 H]PDBu binding to PMN (A), monocytes (B), and lymphocytes (C) by phorbol related compounds [PMA (□), PDBu (○), MZ (●), PDA (△), and PHR (▲)]. In each experiment, specific binding of [3 H]PDBu in the presence of the potential inhibitor is expressed as a percentage of the specific binding in the absence of the competing ligand. Each point represents the value obtained from the mean of three replicates, and the SEM for the triplicates was <5%. All potential inhibitors were tested simultaneously using all three blood cell types obtained from the same donor. Similar results were obtained in three separate experiments. K_i (Table II) were calculated as described (26).

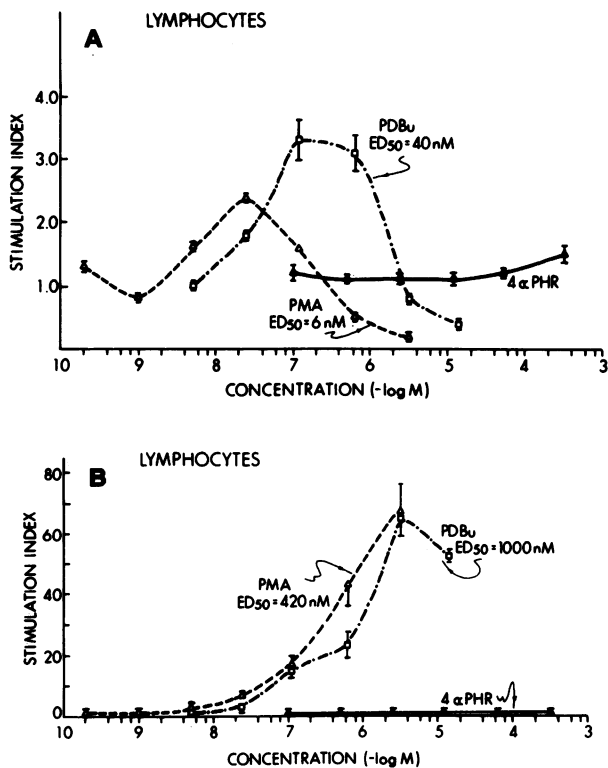


FIGURE 6 Tritiated thymidine incorporation by lymphocytes cultured with phorbol-related compounds in the absence (A) and presence (B) of 10% fetal bovine serum. Data are expressed as stimulation index (that is, sample counts per minute \div control counts per minute). Each point represents the mean of triplicates \pm 1 SEM. This data is representative of two comparable experiments.

thymidine incorporation by lymphocytes, and serotonin release by platelets. Lehrer and Cohen (8) in studies using [³H]PMA and [³H]PDBu demonstrated PMN phorbol diester receptors comparable to those we describe here. They showed that the extent of PMN superoxide production in response to PMA was directly proportional to the fractional occupancy of the receptor by PMA.

Rapid binding of [³H]PDBu to all four cell types occurred at 23° and 37°C, followed by a loss of this cell-associated radioactivity with time (except in the platelet experiments done at 23°C). Binding was slower at 4°C and in most cases reached a maximum plateau level that was stable. These results are consistent with the observations of Solanki and Slaga (28, 29) in mouse epidermal and HL60 cells and Sando et al. (17) with human lymphocytes. The cause of this loss of [³H]PDBu binding is unclear but could result from conformational changes in the binding site with elution of the ligand from the cells. As a steady state of binding was achieved only at 4°C, valid Scatchard

analysis of [³H]PDBu binding data could be made only at this temperature. This analysis was linear for all cell types, indicative of one population of receptor. This is consistent with the findings of Blumberg and colleagues (14, 16, 18) with chicken embryo fibroblasts and mouse brain and skin, and of Shoyab and Todaro and others (15, 17) with a variety of normal and transformed avian and mammalian cells. The K_d calculated from the binding data were similar for all four blood cell types. While the K_d remained relatively constant, the calculated number of binding sites per cell was more variable for an individual cell type in different experiments and between cell types in the same experiment. The causes of this larger variability are not known. The smaller number of binding sites in platelets is consistent with their smaller size.

The absence of specific binding of [³H]PDBu to mature erythrocytes appears to be a unique finding among numerous mammalian and avian cell types. It is important in this regard that platelets, cells also lack-

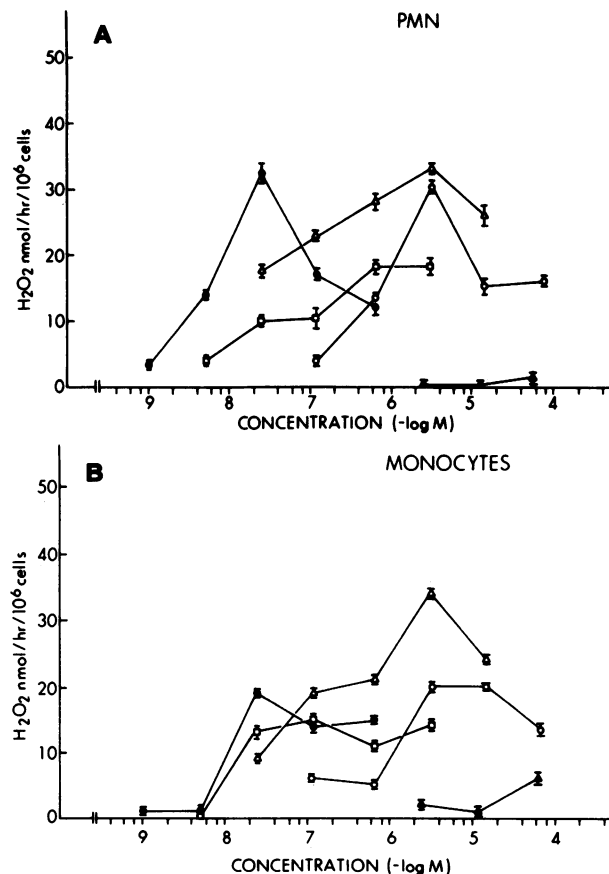


FIGURE 7 H₂O₂ production by PMN (A) and monocytes (B) stimulated by PHR (\blacktriangle), PDA (\circ), PDBu (\square), PMA (\bullet), and MZ (\triangle). All points represent the mean of triplicates \pm 1 SEM. This data is representative of three comparable experiments.

ing a nucleus, have specific receptors for the phorbol esters, and chicken erythrocytes, which are nucleated, show no specific binding of [³H]PDBu (data not shown). Nagle and Blumberg (30) showed that enucleated fibroblasts (like control fibroblasts) displayed morphological changes in response to phorbol diesters. In addition, studies by Dunphy et al. (18), looking at the subcellular localization of [³H]PDBu binding in mouse brain, indicate that the principal site of binding is not the nucleus in that system. All of these factors support the idea that the lack of specific binding of phorbol esters to human erythrocyte is not on the basis of its enucleate state.

The specificity of [³H]PDBu binding to the blood cells studied here was demonstrated by experiments that showed that the biologically active compounds PMA, PDBu, MZ, and PDA inhibited binding while the biologically inactive compounds 4 α -PHR and PHR did not. The relative abilities of these compounds to inhibit binding, as shown by the calculated K_i , paralleled the relative order of their tumor-promoting activities in vivo. In general, the K_d for [³H]PDBu binding correlated well with the ED₅₀ for the various measured functional responses in all the blood cells examined. This is consistent with the concept that the magnitude of these biological responses is directly proportional in a continuous manner for a given cell to occupancy of receptors by the ligand. That is, one would expect a 50% maximal response when 50% of the cellular receptors were occupied by the ligand or when the concentration of free ligand added equaled the K_d for the receptor. Alternatively, as recently suggested for PMA stimulation of PMN H₂O₂ production (31), individual cells could be triggered in an all or none fashion to produce a biological response, with a 50% maximal response occurring when 50% of the total cells were activated. Our data, which are based on responses of populations of cells, does not allow us to discriminate between these two interpretations.

The existence of high affinity receptors for the phor-

TABLE III
ED₅₀ for H₂O₂ Production Stimulated by
Phorbol-related Compounds

Compound	ED ₅₀	
	Monocytes	Lymphocytes
PMA	13 nM	4 nM
PDBu	42 nM	42 nM
MZ	20 nM	20 nM
PDA	850 nM	780 nM
PHR	•	•
4 α -PHR	•	•

• No stimulation noted.

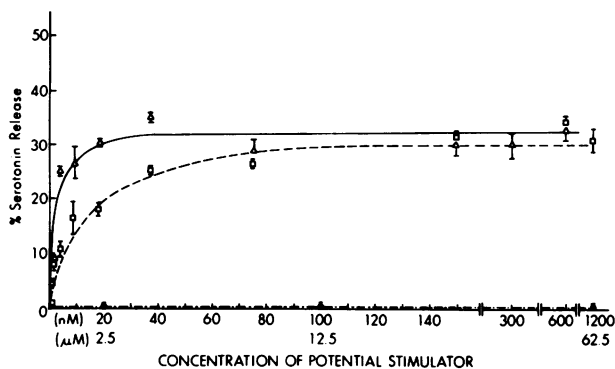


FIGURE 8 [³H]5HT release by platelets in response to phorbol-related compounds. Percent serotonin release was calculated as [(counts per minute in sample supernatant - counts per minute in blank supernatant) ÷ (counts per minute in lysate - counts per minute blank supernatant)] × 100. All points represent the mean of triplicates ± 1 SEM. This experiment is representative of three comparable experiments. Δ , PMA (nM); \square , PDBu; \bullet , PHR and 4 α PHR (μ M).

bol diesters on a variety of animal cells implies the existence of an endogenous ligand for this receptor. Horowitz and Weinstein (32) reported the presence of a factor in serum and tissue extracts that inhibited [³H]PDBu binding to cells. Attempts to isolate and characterize such a ligand that would compete for binding to the receptor with [³H]PDBu and induce comparable cellular effects have not been successful thus far, due in part to the existence of phorbol ester-binding proteins (27) and hydrolases (33), which complicate the search. The many different responses elicited in different normal and leukemic blood cells (3-13, 34, 35) by the phorbol diester ligand-receptor interaction suggests that an endogenous ligand could be responsible for some of the physiologic and pathologic functions noted in these cells. A nontoxic, endogenous ligand for this receptor might have use in experimentally manipulating blood cell function and differentiation [use, e.g., in the experimental treatment of leukemia (35)].

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