Ca²⁺-independent binding of [³H]phorbol dibutyrate to protein kinase C is supported by protamine and other polycations

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The activity of the Ca²⁺- and phospholipid-dependent protein kinase, protein kinase C (PKC), can be modulated by diacylglycerols and phorbol esters. The association of these agents with PKC is, in turn, generally understood to be dependent on Ca²⁺ and phospholipids. Certain substrates, e.g. protamine sulphate, are known to undergo cofactor-independent phosphorylation by PKC. We report here that, in the presence of such substrates, PKC bound 1,2-dihexanoylglycerol and phorbol dibutyrate in a Ca²⁺-independent manner. Histone IIIs, which is phosphorylated by PKC only in the presence of Ca²⁺ and phospholipid, also supported Ca²⁺-independent binding of 1,2-dihexanoylglycerol and phorbol dibutyrate to PKC, but to a lesser extent than did protamine. Support for Ca²⁺-independent binding was also exhibited by non-peptide polycations (e.g. DEAE-cellulose DE52), indicating that recognition of the catalytic site is not a prerequisite for this effect. The natural polyamines spermine and putrescine did not have this property, however. The affinity of PKC for phorbol dibutyrate and 1,2-dihexanoylglycerol was found to be unchanged by the presence of substrates or DE52. It is proposed that, in the absence of Ca²⁺, certain polycations favour expression of the diacylglycerol/phorbol ester binding site by stabilizing the active conformation of PKC.

INTRODUCTION

Protein kinase C (PKC) is believed to play a central role in control of cell function (for reviews see [1–4]). The activity of this enzyme is commonly regarded as being fully dependent on Ca²⁺ and a negatively charged phospholipid [e.g. phosphatidylserine (PS)], and it can be further modulated by diacylglycerol (DAG). This second messenger, which is probably derived from phospholipase C degradation of membrane phospholipids, acts by greatly increasing the affinity of PKC for the other activators [4–8]. Thus the concentration of DAG, which is increased by cell-activating mediators and growth factors, exerts a physiological control over the activity of PKC. Many of the cellular actions of DAGs are mimicked by the tumour-promoting phorbol esters [9–12], which share a common binding site on PKC [13–15]. Like PKC activation, phorbol ester binding to the isolated enzyme has been shown to be dependent on Ca²⁺ and phospholipid [16–18].

Although DAG concentrations control PKC activity under many conditions [4–8], there are at least two mechanisms whereby PKC activation can occur, *in vitro*, in a DAG-independent manner. For example, the proteolytic activation of PKC is well established [19–21]. However, the significance of this as a physiological mechanism for control of PKC in whole cells is unknown [22]. Because of the probable influence of PKC in control of cellular functions, it is important that the physiological significance of any modulatory pathway can be defined.

The present report focuses on the mechanism whereby the phosphorylation of certain substrates by isolated PKC occurs independently of DAG, Ca²⁺ and PS. For example, protamine sulphate is phosphorylated in the

absence of any of the known cofactors [20,23], whereas the phosphorylation of a different group of substrates [e.g. myelin basic protein and the synthetic polypeptide poly(Arg₃,Ser)] requires PS but not Ca²⁺ [24]. The mechanism whereby a substrate can induce PKC activity in the absence of PS, DAG or Ca²⁺ is not known. Furthermore, it is not clear whether such activity has physiological significance.

We report here that the presence of substrates that induce DAG-independent phosphorylation caused a change in the enzyme that allowed DAG and [³H]PDBu to bind to PKC in a Ca²⁺-independent manner. This change was also brought about by polycationic ion-exchange resins. The implications of these observations for the mechanism whereby PKC is activated and the possible physiological relevance of Ca²⁺-independent binding of DAG are discussed.

MATERIALS AND METHODS

Isolation of protein kinase C

PKC was isolated from rat brain by DEAE-cellulose chromatography as described previously [17]. The preparation contained several different proteins, but was enriched with PKC, as judged by its histone-phosphorylating activity, which was dependent on Ca²⁺ and PS and stimulated 8–15-fold by addition of 1,2-dihexanoyl-*sn*-glycerol (HHG), activation being specific for the 1,2-*sn*-enantiomer.

[³H]PDBu binding assay

(i) **In PS/PC mixtures.** Phosphatidylcholine (PC), PS, PDBu, [³H]PDBu (New England Nuclear) and HHG as

Abbreviations used: PDBu, phorbol 12,13-dibutyrate; DAG, diacylglycerol; HHG, 1,2-dihexanoyl-*sn*-glycerol; PKC, protein kinase C; PS, phosphatidylserine; PC, phosphatidylcholine.

required were dried down under a stream of N_2 and made up to 10 times their final concentration in sonicating buffer, containing 250 mM-Tris/HCl and 2 mM-EGTA, pH 7.50 at 27 °C. Unless otherwise indicated, the final concentrations were 20 μ M-PS, 80 μ M-PC and 10 nM-[3 H]PDBu (8–15 Ci/mmol). Non-specific binding was determined in the presence of 10 μ M unlabelled PDBu, and specific binding was calculated as the difference between total and non-specific binding. After incubation at 27 °C for 5–10 min the lipid mixture was mixed well, then sonicated in a water bath (Sonicor) for 1–2 min. At this stage the lipid preparation still retained some cloudiness, and by the criteria of Boni & Rando [25] probably consisted of a mixture of multilamellar vesicles. The enzyme was prepared in 25 mM-Tris/HCl/0.1 mM-EGTA (pH 7.50 at 27 °C). The assay buffer contained 25 mM-Tris/HCl and 6.25 mM-EGTA (pH 7.50 at 27 °C), with $CaCl_2$ and $MgCl_2$ added to give the final calculated free cation concentrations shown in the Results and discussion section [26]. A 200 μ l portion of assay buffer was prewarmed to 27 °C, and the assay was started by adding 25 μ l of lipid mixture and 25 μ l of enzyme in quick succession. After 1 min at 27 °C, 200 μ l of the mixture was removed and filtered through a GF/C filter (Whatman), followed by three 4 ml washes with 25 mM-Tris/HCl/2.5 mM- $MgCl_2$ /100 μ M- $CaCl_2$, pH 7.5 at 4 °C. The filters were then removed and dispersed in 5 ml of scintillant (Pico-fluor, Packard) before 3 H associated with the filters was assessed in a Beckman scintillation counter.

(ii) **In PS/Triton mixed micelles.** The detergent micelle system was adapted from that described by others [27], but the binding assay was performed essentially as described for PS/PC vesicles, except that the dried lipids were taken up in sonicating buffer containing 3 % Triton X-100. PC was omitted from these detergent micelles, and the final concentration of PS was 1.1 mM (20 mol % of total micelle content).

Effect of polycations on binding

The effect of the PKC substrates on [3 H]PDBu binding was determined by dissolving them in the assay buffer. The particulate ion exchangers were prepared by extensive washing in a column with the relevant assay buffer before preparing a suspension at 0.12 g/ml (pre-swollen weight).

Determination of phospholipid recovery

The recovery of PS on the GF/C filter was determined by incorporating L-3-phosphatidyl-L-[U- 14 C]serine (Amersham International, Amersham, Bucks., U.K.) into the PS/PC mixtures to a final specific radioactivity of 0.5 Ci/mmol. 14 C associated with filters was assessed as described for 3 H above.

Materials

PS (Na^+ salt) was obtained from Lipid Products (Redhill, Surrey, U.K.). PC, PDBu, Triton X-100, protamine sulphate, histone type IIIs and poly(Arg₃,Ser) were obtained from Sigma. EGTA (puriss p.a. grade) was obtained from Fluka. HHG was prepared as described previously [28]. All other reagents were of analytical grade.

Fig. 1. Binding of [3 H]PDBu to isolated PKC: dependence on free Ca^{2+} concentration

[3 H]PDBu binding to PKC was determined in PS/PC phospholipid vesicles as described in the Materials and methods section. The free Ca^{2+} concentration was set by using 5 mM-EGTA- Ca^{2+} buffers and calculated in accordance with Fabiato & Fabiato [26]. Points are means of triplicate determinations from one experiment, which is representative of several others.

RESULTS AND DISCUSSION

Ca^{2+} -independent binding supported by substrates

Ligand binding to the DAG site on PKC has invariably been measured in the absence of substrates, and as a result the possible effect of different substrates on this modulatory site is unknown. To gain some insight into the mechanism by which certain substrates activate the enzyme in the absence of DAG and Ca^{2+} , we have measured [3 H]PDBu binding to PKC in the absence and the presence of selected substrates. This has revealed dramatic effects on at least one aspect of [3 H]PDBu binding, the Ca^{2+} -dependence. Fig. 1 demonstrates that the binding of [3 H]PDBu to PKC in the absence of substrate was dependent on Ca^{2+} . The phospholipid requirement was provided by a crude sonicated preparation of 20 mol % PS in PC. Although only a small amount of specific binding was observed in the absence of Ca^{2+} (< 10 % of maximum), the value increased dramatically as the free Ca^{2+} concentration was increased over the physiological range (0.1–10 μ M). PKC substrates chosen from the three different categories proposed by Ferrari *et al.* [23], namely histone IIIs, poly(Arg₃,Ser), poly(Lys₃,Ser) and protamine sulphate, all increased the [3 H]PDBu binding measured in the absence of Ca^{2+} (5 mM-EGTA) (Table 1). In addition, protamine, poly(Arg₃,Ser) and poly(Lys₃,Ser) tended to increase the binding observed in the presence of 10 μ M- Ca^{2+} . The degree of potentiation of binding caused by the substrates was variable. In some experiments (results not shown), protamine increased binding of [3 H]PDBu by as much as 4-fold. Although we were unable to trace the factor(s) responsible for this variability, there was no doubt that Ca^{2+} -independent binding supported by substrates was observed in all experiments. It is noteworthy that protamine, which is phosphorylated in the absence of Ca^{2+} and PS, and poly(Arg₃,Ser) and poly(Lys₃,Ser) which require PS but not Ca^{2+} , supported more Ca^{2+} -independent binding than did histone IIIs, which requires both PS and Ca^{2+} for phosphorylation by PKC. Removal of Mg^{2+} as well as Ca^{2+} did not decrease

Table 1. Effect of substrates on [³H]PDBu binding to PKC in the presence and the absence of bivalent cations

Binding was assessed in PS/PC phospholipid vesicles as described in the Materials and methods section, in which bivalent-cation concentrations were controlled by using 5 mM-EGTA buffers in accordance with Fabiato & Fabiato [26]. Data (means $\pm$ s.d.) are drawn from the numbers of experiments shown in parentheses, and are expressed as a percentage of the binding observed in the presence of Ca²⁺ (10 μ M) and Mg²⁺ (2.5 mM) but in the absence of added polycations; N.D., not determined.

Substrate	Conditions ...	Binding (%)		
		Full assay	– Ca ²⁺	– Ca ²⁺ – Mg ²⁺
None		100	12 $\pm$ 5 (7)	4 $\pm$ 4 (3)
+ Protamine (0.1 mg/ml)		135 $\pm$ 20 (7)	127 $\pm$ 29 (7)	234 $\pm$ 8 (3)
+ Poly(Arg ₃ ,Ser) (0.1 mg/ml)		135 $\pm$ 56 (4)	113 $\pm$ 35 (4)	142 $\pm$ 47 (3)
+ Poly(Lys ₃ ,Ser) (0.1 mg/ml)		129 $\pm$ 32 (3)	118 $\pm$ 33 (3)	N.D.
+ Histone III _s (1 mg/ml)		97 $\pm$ 5 (4)	40 $\pm$ 8 (4)	N.D.

Table 2. Effect of cations and polycations on [³H]PDBu binding in the presence and the absence of Ca²⁺ or Mg²⁺

Details are as in Table 1 for PS/PC vesicle assay. A and B refer to the monoamines *NN*-diethylaminoethanol and *N*-(2-benzyloxyethyl)-*NN*-diethylamine respectively. [³H]PDBu binding to PKC in PS/Triton X-100 mixed micelles was performed as described in the Materials and methods section. Data represent means $\pm$ s.d. from the numbers of experiments shown in parentheses; N.D., not determined.

Polycation	Conditions ...	Binding (%)		
		Full assay	– Ca ²⁺	– Ca ²⁺ – Mg ²⁺
PS/PC, no additions		100	12 $\pm$ 5 (7)	4 $\pm$ 4 (3)
PS/PC + DE52 (0.12 g/ml)		131 $\pm$ 16 (3)	56 $\pm$ 7 (3)	51 $\pm$ 11 (3)
PS/PC + polylysine (0.1 mg/ml)		117 $\pm$ 14 (3)	102 $\pm$ 27 (3)	N.D.
PS/PC + 0.63 M-A		101, 102 (2)	9 $\pm$ 4 (3)	N.D.
PS/PC + 0.63 M-B		122, 63 (2)	1 $\pm$ 8 (3)	N.D.
PS/PC + 1 mM-spermine		102 $\pm$ 18 (4)	19 $\pm$ 6 (4)	N.D.
PC/PC + 1 mM-putrescine		89 $\pm$ 18 (4)	12 $\pm$ 4 (4)	N.D.
PS/Triton X-100 + DE52 (0.12 g/ml)		100*	69 $\pm$ 3 (3)*	N.D.

* Binding in the PS/Triton X-100 micelles is expressed as a percentage of that observed in the presence of DE52 as well as Ca²⁺ and Mg²⁺ (see the text for further details).

the Ca²⁺-independent binding supported by any of the substrates (Table 1), indicating that the remainder of the binding was independent of all bivalent cations. Binding was, in all cases, fully dependent on the presence of PKC, demonstrating that it did not result from direct association of [³H]PDBu with the substrate (results not shown).

Ca²⁺-independent binding supported by other polycations

To investigate whether these properties were specific to polyamine-peptide substrates, we have assessed the effect of peptidic and non-peptidic polycations on [³H]PDBu binding to PKC in PS/PC mixed vesicles. The non-substrate polylysine was equipotent with poly(Lys₃,Ser) at supporting Ca²⁺-independent binding. The DEAE-cellulose polymer DE52 (Whatman) was less effective than polylysine, but still supported a significant degree of Ca²⁺-independent binding, and also potentiated the binding observed in the presence of Ca²⁺ (Table 2). Ca²⁺-independent binding was also observed in the presence of DEAE-agarose (Bio-Rad) and two polycationic resins [IR45 (BDH); Dowex 1-X2 (Bio-Rad)] (results not shown). As with the polypeptides, the Ca²⁺-independent binding supported by DE52 was independent of the

presence of Mg²⁺ (Table 2) and fully dependent on the presence of enzyme (results not shown). The natural polyamines spermine and putrescine did not support Ca²⁺-independent binding (Table 2). If the concentration of polyamine was increased from 1 mM to 10 mM, a decrease in specific binding was observed. It therefore seems that the Ca²⁺-independent binding observed here is distinct from the inhibitory effects of polycations on PKC observed previously [29,30].

The effect of polycationic polymers was not peculiar to PS/PC mixed vesicles, since binding of [³H]PDBu to PKC in PS/Triton X-100 mixed micelles was Ca²⁺-independent either when bound ligand was isolated by using DEAE-cellulose filters (results not shown) or when DE52 was present in the assay and bound ligand was separated with GF/C filters (Table 2). With these detergent micelles the GF/C filters did not retain any bound ligand in the absence of DE52, whether Ca²⁺ was present or not. Since [³H]PDBu has been shown to associate with PKC in PS/Triton mixed micelles [18], it seems that the detergent prevented the interaction between the micelles and the GF/C filter. The retention of bound [³H]PDBu by GF/C filters in the presence of DE52 probably depends on the ionic interaction of PS/

Table 3. Effect of polycations on [¹⁴C]PS recovery on GF/C filters

[¹⁴C]PS was incorporated into PS/PC phospholipid vesicles used for the [³H]PDBu-binding assay, and recovery was determined as described in the Materials and methods section. The assay was performed in the presence or absence of Ca²⁺ (10 μ M) as indicated. Data are means $\pm$ S.D. for the numbers of experiments indicated in parentheses.

Addition	¹⁴ C-PS recovered on filter (% of total added)	
	+ Ca ²⁺	No Ca ²⁺
None	42 $\pm$ 10 (7)	39 $\pm$ 8 (7)
Protamine (0.1 mg/ml)	72 $\pm$ 3 (7)	71 $\pm$ 4 (7)
Poly(Arg ₃ ,Ser) (0.1 mg/ml)	72 $\pm$ 3 (3)	69 $\pm$ 4 (3)
Poly(Lys ₃ ,Ser) (0.1 mg/ml)	73 $\pm$ 1 (3)	71 $\pm$ 2 (3)
Histone (0.1 mg/ml)	55 $\pm$ 9 (4)	55 $\pm$ 11 (4)
Polylysine (0.1 mg/ml)	70 $\pm$ 3 (3)	69 $\pm$ 5 (3)
DE52 (0.12 g/ml)	62 $\pm$ 2 (3)	59 $\pm$ 10 (3)

Triton/PKC/[³H]PDBu complexes with the relatively large particles of resin.

Ca²⁺- and PS-independent phosphorylation by PKC requires the presence of a number of arginine residues flanking the phosphorylation site on the substrate [23,32]. However, since polylysine and a variety of DEAE polymers can mimic the effects of such substrates, it appears that augmentation of binding does not result from the recognition of substrate by the catalytic site. Replacement of Ca²⁺ by the polycation was considered a possibility. However, this is unlikely, since the monoamines *NN*-diaminoethanol and *N*-(2-benzyloxyethyl)-*NN*-diethylamine were unable to support Ca²⁺-independent binding in PS/PC mixed vesicles (Table 2, 'A' and 'B' respectively).

The additional binding supported by the polycations in both the presence and the absence of Ca²⁺ could result either from an increase in the number of binding sites available to [³H]PDBu or from an increase in the affinity of the existing sites. The latter possibility was tested by measuring the IC₅₀ values for displacement of [³H]PDBu, either by unlabelled PDBu or by HHG, in the presence and absence of polycations. In the absence of polycations the IC₅₀ values (concn. giving 50% inhibition; mean $\pm$ S.D.) were for PDBu 17.3 $\pm$ 3.5 nM (*n* = 6) and for HHG 18.3 $\pm$ 7.6 μ M (*n* = 3). These values were largely unchanged by addition of protamine or DE52, either in the presence or the absence of Ca²⁺ (results not shown). Thus the increased binding observed in the presence of polycations appears to be due to an increase in the number of binding sites. One exception to this appears to have been when HHG (up to 100 μ M) failed to displace [³H]PDBu bound to PKC (in PS/PC vesicles) in the presence of DE52 and in the absence of Ca²⁺. The precise reason for this is unclear, but it did not arise from irreversible binding of [³H]PDBu, as the ligand was fully displaced by unlabelled PDBu (IC₅₀ = 15.3 $\pm$ 5.5 nM). Furthermore, HHG displaced [³H]PDBu from PKC in PS/Triton micelles in the presence of DE52 (IC₅₀ = 24.6 $\pm$ 11.7 μ M), indicating that the effectiveness of HHG as a ligand was unchanged by the presence of this

polycation. Thus there appears to have been a unique interaction between the three components (PS/PC vesicles, HHG and DE52), which decreased the effectiveness of HHG as a displacing ligand in the absence of Ca²⁺.

Effect of the polycations on the retention of [¹⁴C]PS-labelled PS/PC vesicles on GF/C filters

The increase in specific binding of [³H]PDBu in the presence of certain polycations may indicate that an additional class of DAG-binding sites is harnessed from the crude PKC preparation by the polycations. However, a number of observations suggested that this might not be the case. Firstly, there was no evidence of heterogeneity in the curves for displacement of [³H]PDBu by either HHG or PDBu in the presence of polycations. Secondly, since the effect of substrates on [³H]PDBu binding was not additive with the effect of Ca²⁺ (Table 1), it appeared that these agents supported binding to a common population of sites. The additional binding could have resulted from an increased recovery of PKC on the GF/C filters, thereby increasing the number of binding sites. We have examined this hypothesis by measuring the recovery of [¹⁴C]PS/PC vesicles during the binding assay. This provided a simple means of assessing PKC recovery, assuming that PKC associated randomly with phospholipid vesicles in suspension.

When [¹⁴C]PS/PC micelles, suspended in Ca²⁺ buffer and in the absence of polycation, were passed through GF/C filters, only 40% of the [¹⁴C]PS was retained (Table 3). The recovery of [¹⁴C]PS was increased substantially by the addition of substrates or polycations. Protamine and the polyarginine and lysine peptides were more effective than were histone and DE52. The increased recovery of [¹⁴C]PS was probably due to the polyvalent amines cross-linking the negatively charged phospholipid vesicles/micelles, causing them to be retained by the GF/C filter through increased size and/or increased ionic interaction with the filter.

In order to assess the true effect of Ca²⁺ and polycations on binding of [³H]PDBu to PKC, the proportional increase in binding of [³H]PDBu was corrected for the increase in PKC recovery estimated from the retention of [¹⁴C]PS under the same experimental conditions. When this correction was made, it became evident that a significant proportion of the increased binding of [³H]PDBu brought about by addition of polycations was due to increased retention of PS/PC vesicles, and hence PKC, by the GF/C filters (Fig. 2). Interpretation of the effects of these agents on [³H]PDBu binding in the absence of Ca²⁺ also became clearer. Fig. 2 shows that protamine, poly(Arg₃,Ser), poly(Lys₃,Ser), polylysine, histone and DE52 all supported Ca²⁺-independent binding that could not be accounted for by increased retention of PKC in the filter. Protamine was the most effective, supporting binding to the same extent, in both the presence and the absence of Ca²⁺. The polyarginine and lysine peptides were all equipotent at supporting Ca²⁺-independent binding, but as a group were slightly less effective than protamine. The small amount of Ca²⁺-independent binding supported by histone IIIs and DE52 was observed consistently. Results shown in Fig. 2 suggest that some of the polypeptides inhibit [³H]PDBu binding in the presence of Ca²⁺, but this has not been explored further.

From the data presented here, it is reasonable to

Fig. 2. [³H]PDBu binding corrected for phospholipid recovery

[³H]PDBu binding (▨) in PS/PC vesicles was corrected for [¹⁴C]PS recovery; □, uncorrected data from Tables 1 and 2 for comparison. Data are expressed as a percentage of binding observed in the presence of Ca²⁺ but in the absence of added polycation (▨). Data are means ± S.D. for three experiments.

suggest a link between the capacity of a substrate to support Ca²⁺-independent binding and to be phosphorylated by PKC in the absence of other cofactors. Cofactor-independent phosphorylation relies on a specific positioning of the basic residues in relation to the site of phosphorylation [23,32]. The augmentation of binding, on the other hand, was observed with a range of polybasic agents, although the greater effectiveness of protamine remains to be explained. The apparent anomaly whereby histone IIIs appeared to support a small degree of Ca²⁺-independent binding of [³H]PDBu, but is not phosphorylated at all in the absence of Ca²⁺ [27], is consistent with its extremely basic properties. It is not unreasonable to propose that polycations stabilize the active conformation of PKC in which the DAG site is 'open'. The same conformational change would normally be induced by DAG, but only in the presence of Ca²⁺ and PS. The observation that a peptide capable of undergoing Ca²⁺- and PS-independent phosphorylation by PKC cannot support the phosphorylation of one that is not [23] implies a strict positional relationship between the active site and the polycation site(s), such that a bound polycation will block further access of another substrate to the active site. This interpretation indicates that polycations should act as inhibitors of PKC. Inhibition has, in fact, been reported for high concentrations of some polycations, e.g. spermine and putrescine [29,30]. However, for spermine and putrescine the mechanism of interaction with PKC seems to be different from that proposed here (Table 2).

Protamine is phosphorylated by PKC in the absence of PS as well as in the absence of Ca²⁺ [19–21]. However, the GF/C filter technique is not suitable as a means of detecting PS-independent [³H]PDBu binding, because the negatively charged phospholipid probably plays an important role in retention of phospholipid vesicles and the associated PKC–ligand complex by the GF/C filter. For this reason, the PS-dependence of [³H]PDBu binding to PKC was not evaluated in the present study.

The water-soluble form of PKC does not express the hydrophobic region containing the DAG-binding site and is inactive. Activation of the enzyme by any of the known mechanisms is associated with expression of this hydrophobic region or domain and the DAG-binding site. These include the action of DAG in the presence of PS and Ca²⁺, the action of protamine described here and also proteolytic cleavage by trypsin [33]. This relationship may have important implications in the mechanism of action of the regulatory domain.

The polycation-binding site(s) on PKC could represent an important modulatory site(s), as it appears to be associated with both activation and inhibition of the isolated enzyme. It is unclear, however, if this sort of modulation has any importance in the whole cell. Likewise the physiological role of DAG binding supported by polycations, as distinct from binding supported by Ca²⁺, is also uncertain. If appropriate polycations have access to PKC in whole cells, the enzyme would be fully active without the need for further modulations by DAG. A role for DAG in modulating

the possible inhibition of PKC by natural polyamines cannot be deduced from our present knowledge.

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