0031-6997/89/4102-0211$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 41, No. 2
Printed in U.S.A.

Modulation of Ion Channel Activity: A Key Function of the Protein Kinase C Enzyme Family

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I. Introduction

RECEPTOR-MEDIATED hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2)† is a commonly utilized mechanism for transducing the information from various extracellular signals into cells. The immediate products of this catalytic reaction, inositol 1,4,5-trisphosphate (1, 4, 5-IP_3) and a 1,2-*sn*-diacylglycerol (DAG) act as the second messenger molecules at the beginning of a bifurcating signal transduction pathway, involving calcium (Ca^{2+}) mobilization from intracellular storage pools, and the activation of the protein kinase C (PKC) enzyme family, respectively (25, 113, 169–171). This appears to be the primary mechanism for initiating PKC-mediated cellular effects, although it is apparent that alternative pathways may be utilized for generating the necessary Ca^{2+} signal (either through a ligand- (136) or voltage-operated channel) and a DAG, or alternative lipid activator such as arachidonic acid. (For example, by a Ca^{2+}-dependent or guanine nucleotide binding protein (G-protein)-mediated activation of phospholipases C or A_2.) Physiological activation of PKC by DAG can be mimicked by tumor-promoting phorbol esters, such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA), for which it appears to be the cell surface receptor (11, 38, 112, 168).

PKC occupies a pivotal position in the biochemical pathways that relay information into the cell, because it is able to influence the cellular response to numerous other stimuli. These include those using receptors coupled to PIP_2 hydrolysis, as well as growth factor receptors and those coupled to the activation of cyclic nucleotide-dependent protein kinases. This control may be exerted at different levels of the signaling pathways, with either a positive or negative resultant effect (171). The formation of an active enzyme complex at the plasma membrane, by the coordinate action of phosphatidylserine (PtdSer), a DAG, and Ca^{2+} has always implied that the primary targets of PKC are membrane proteins. In this respect, it is becoming clear that a key function of the enzyme is its ability to modulate the movement of certain ions into and out of the cytosolic compartment of the cell, and thus to regulate the cellular processes that depend on this. Alteration of the activity of 'exchange' and 'pump' proteins by PKC is well documented (170). Recently, a wealth of information has become available that demonstrates that PKC is able also to modulate the activity of the numerous, pharmacologically distinct types of ion channels that are expressed in most, if not all, cells. The involvement of these ion channels in a variety of key cellular processes necessitates a detailed understanding of their activity and its modulation. In this article, we summarize the known properties of the PKC enzyme family as it now stands, discuss some general properties of ion channel proteins and how these appear to be altered by protein kinases, and then present a comprehensive account of the reported effects of PKC activation on ion channel activity. Literature up to the end of February 1989 has been included.

A. Protein Kinase C Enzyme Family

1. Structural heterogeneity. Four complementary DNA (cDNA) clones encoding the α-, βI-, βII-, and γ-subspecies of PKC‡ were reported initially in bovine (188, 47), rat (119, 141, 178, 179, 114, 115, 92, 183), rabbit (175, 176), and human (47,48) brain libraries. Subsequent to this, the βI- and βII-PKC clones were reported in a human spleen cDNA library (122), and the α-PKC from a mouse Swiss 3T3 cDNA library (200). The genes for the α-, β-, and γ-subspecies are known, at least in humans, to reside on different chromosomes (47), whereas the closely related βI- and βII-subspecies are derived from a single gene by alternative splicing of the 3-primed exons (181, 48, 123). Another group of cDNA clones, which encode the δ-, ϵ-, and ζ-subspecies were isolated recently from a rat brain library (182, 184). In addition, a partial nucleotide sequence of a rat brain cDNA clone encoding a polypeptide nearly identical to part of ϵ-PKC ('RP16'; 92), and cDNA clones encoding the ϵ-subspecies from a rabbit brain library ('nPKC'; 177) and mouse brain library (205) have been reported.

The PKC subspecies encoded by the above clones can be grouped according to their structural characteristics (fig. 1). The α-, βI-, βII-, and γ-subspecies consist of a single polypeptide chain having four regions of highly conserved sequence (C1-C4) interspersed with five regions of variable sequence (V1-V5). The length of the polypeptide chains, and the molecular mass of the rat subspecies, predicted from their deduced amino acid sequences, are given in table 1. The βI- and βII-subspecies are identical, apart from a stretch of approximately 50 amino acid residues in the region C4/V5, which contains only 50% sequence homology.

The conserved region C1 contains a tandem repeat of

† Abbreviations used are: AHP, after-hyperpolarization; $[Ca^{2+}]_i$, intracellular calcium concentration; CCK-8, cholecystokinin octapeptide 26–33; cDNA, complementary DNA; ω-CgTx, ω-conotoxin GVIA; DAG, 1,2-diacylglycerol; DHP, 1,4-dihydropyridine; DiC_8, 1,2-dioctanoylglycerol; DiC_{10}, 1,2-didecananoylglycerol; DPBA, 12-deoxyphorbol 13-isobutyrate 20-acetate; DRG, dorsal root ganglion; GABA, γ-aminobutyric acid; GnRH, gonadotropin-releasing hormone; H-7, 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine hydrochloride; $I_{Ca()}$, a species of calcium current; $I_{K()}$, a species of potassium current; 1,4,5-IP_3, inositol 1,4,5-triphosphate; LHRH, luteinizing hormone-releasing hormone; LTP, long-term potentiation; mRNA, messenger RNA; nACh, nicotinic acetylcholine; NMDA, *N*-methyl-D-aspartate; NPY, neuropeptide Y; OAG, 1-oleoyl 2-acetylglycerol; PDA, 4β-phorbol 12,13-diacetate; PDBu, 4β-phorbol 12,13-dibutyrate; 4α-PDD, 4α-phorbol 12,13-diacetate; 4β-PDD, 4β-phorbol 12,13-diacetate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PtdSer, phosphatidylserine; TEA, tetraethylammonium chloride; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; TRH, thyrotropin-releasing hormone.

‡ The nomenclature for the PKC subspecies used in this article is the same as in previous publications from this laboratory. The interrelationship of the PKC clones reported from the molecular cloning analysis of various groups has been described by Ono & Kikkawa (180).

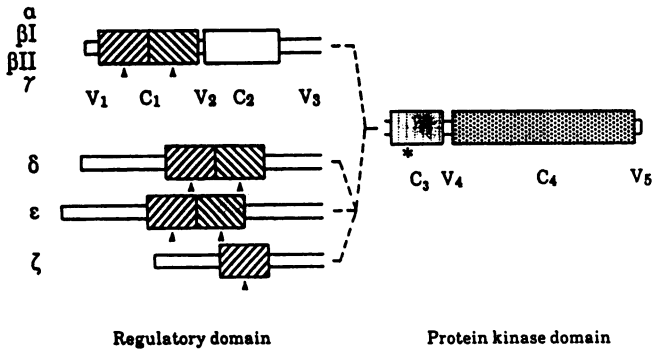

FIG. 1. Schematic representation of the predicted primary structures of the seven subspecies of protein kinase C identified in rat (mammalian) brain. The $\alpha/\beta I/\beta II/\gamma$-group of subspecies consist of a single polypeptide chain having four highly conserved (C1-C4) and five variable (V1-V5) regions. The *N*-terminal half of the molecules form the regulatory domains, with the region C1 containing a repeat of a cysteine-rich sequence (arrow heads) that resembles the consensus sequence of a 'cysteine-zinc DNA-binding finger'. The *C*-terminal half of the molecules form the protein kinase domain, with the region C3 containing an ATP-binding sequence (asterisk). The $\delta/\epsilon/\zeta$-group of subspecies have a protein kinase domain homologous to the $\alpha/\beta I/\beta II/\gamma$-group, but an altered regulatory domain. Notably, the region C2 (open box) is absent, and the ζ-subspecies possesses only one cysteine-rich sequence in the region C1.

a cysteine-rich sequence (position indicated by arrowheads in fig. 1), Cys-X_2-Cys-$X_{13(14)}$-Cys-X_2-Cys-X_7-Cys-X_7-Cys (where X represents any amino acid), that resembles the consensus sequence of a 'cysteine-zinc DNA-binding finger' (24). A similar sequence is also found in the proto-oncogenes c-*raf* and A-*raf* (104, 28). Deletion mutation studies have shown that within the PKC molecule, this stuctural motif is essential for phorbol ester binding (264). The conserved region C2, together with C1, make up the regulatory domain, which confers the overall Ca^{2+}, DAG- and phospholipid-binding properties on the PKC molecules. The conserved regions C3 and C4, present in the carboxyl (*C*)-terminal half of the molecule, form the protein kinase domain, and have large clusters of sequence homology with other protein kinases (81). An ATP-binding sequence, Gly-X-Gly-X-X-Gly—Lys (where X represents any amino acid; 100), is present in the region C3 (position indicated by an asterisk). The two domains can be separated by chromatographic techniques, after limited proteolysis at the region V3 with the calcium-dependent neutral protease, calpain (116).

The δ-, ϵ-, and ζ-subspecies also consist of a single polypeptide chain, but are structurally less similar to one another and to the $\alpha/\beta I/\beta II/\gamma$-group of subspecies (fig. 1). Most notably, the regulatory domain lacks the region C2, and contains extended nonuniform stretches of variable sequence. The region C1 of the δ- and ϵ-PKCs contains the tandem repeats of cysteine-rich sequence, whereas the ζ-PKC contains only one such sequence, and has a relatively low molecular mass (261; table 1). The $\delta/\epsilon/\zeta$-group has the characteristic protein kinase domain, and contains an ATP-binding sequence, although the ζ-subspecies has the glycine residue at position 264 replaced by an alanine (184).

2. Enzymological properties. The diversity of amino acid sequences in the variable regions of the $\alpha/\beta I/\beta II/\gamma$-group of PKC subspecies has allowed the purified or partially purified enzymes from rat (95–97, 101, 114, 181, 209, 210, 212, 214, 12, 189, 120), rabbit (106, 84, 62), monkey (96), bovine (215, 217), human (216, 235, 26), and mouse (K. Ogita and M. S. Shearman, unpublished observations) tissues to be resolved into subfractions by chromatography on hydroxyapatite columns. The structure and genetic identity of the subfractions isolated from brain tissue was first determined by comparison with the enzymes expressed in, and subsequently purified from, COS 7 cells, after transfection with plasmids containing cDNA inserts encoding the individual enzymes (114, 181). The conclusions drawn from these studies were confirmed independently by immunoblotting analysis, using type-specific monoclonal antibodies, of COS 7 cells transfected with plasmids containing cDNA inserts of the different PKC subspecies (97). Three subfractions isolated from brain tissue, designated type I, II, and III (on the basis of their elution pattern from the hydroxy-

TABLE 1
Subspecies of protein kinase C from rat (mammalian tissues)

Subspecies	α	βI	βII	γ	δ	ε	ζ
Amino acid residues	672	671	673	697	673	737	592
Deduced molecular mass (Daltons)	76 799	76 790	76 933	78 366	77 517	83 474	67 740
Chromosome location (human*)	17	16		19	?	?	?
Hydroxyapatite chromatographic subfraction	type III	type II		type I	Not identified	Not identified	Not identified
Tissue expression	Ubiquitous	Some tissues & cells	Many tissues & cells	CNS tissue only (?)	Many tissues	Limited	Limited
Characteristic feature of activation†	High arachidonic acid in the presence of Ca^{2+} ions	PtdSer/DAG in the absence of Ca^{2+} ions		Low arachidonic acid in the absence of Ca^{2+} ions	PtdSer/DAG activation is insensitive to the Ca^{2+} ion concentration		

* Data from Ref. 47
† With H1 histone as the phosphate acceptor.

apatite column) correspond to the γ-, β-(βI and βII), and α-subspecies, respectively§ (table 1). PKC subspecies with βI- and βII-sequence show nearly identical kinetic and catalytic properties, but can be distinguished from each other with type-specific polyclonal antibodies raised against unique peptide sequences present in the region C4/V5 of the molecules (212, 9). The PKC subspecies fractionated in this way exhibit subtle differences in their mode of activation and in their autophosphorylation (95, 98, 248, 106, 209, 210, 189). In our hands, with H1 histone as a substrate, both Ca^{2+} and PtdSer are required for the activation of all three subspecies, and in the presence of a DAG or active phorbol ester isomer, maximal activity is attained at lower Ca^{2+} concentrations. For the type II(β) enzymes, significant activity can be attained with PtdSer and a DAG in the absence of Ca^{2+}. In addition to the slight differences in activation by phospholipids, certain unsaturated fatty acids and arachidonic acid and some of its lipoxygenase metabolites, such as lipoxin A, have been shown to differentially activate the subspecies (209, 210, 163, 217). Type II(β) and type III(α) enzymes respond to increasing concentrations of arachidonic acid (up to 800 μM) in a Ca^{2+}-dependent manner, whereas the type I(γ) enzyme shows a biphasic response, with maximum activity occurring at low concentrations (10–20 μM), and higher concentrations becoming less effective. A significant component of the activation of the latter subspecies by unsaturated fatty acids can be achieved independently of Ca^{2+} (table 1). It should be noted, however, that the absolute requirements for enzyme activation can vary greatly, depending both on the assay system employed (for example, compare 210 and 98), and on the phosphate acceptor (18).

The rat δ/ε/ζ-group of subspecies, expressed in COS 7 cells and then partially purified, have been found to exhibit a different mode of activation with Ca^{2+}, PtdSer and DAG, when compared to the α/βI/βII/γ-group. Partial activation can be achieved in the absence of lipid activators, yet all three components are needed for full activity (184, 261). The rabbit ε-subspecies shows a similar activation, can bind 4β-phorbol 12,13-dibutyrate (PDBu) with high affinity, and also undergoes autophosphorylation (177). Characterization of the partially purified mouse ε-subspecies expressed in COS 1 cells, using a peptide substrate synthesized on the basis of the putative pseudosubstrate sequence, has suggested that it may possess a distinct specificity in the recognition of its substrate (205). The different activation requirements of this group of subspecies seems to stem from the altered regulatory domain structure, particularly the absence of the region C2.

3. Distribution. Investigation of the distribution of PKC in mammalian tissues has been approached using a number of complementary techniques. Enzymatic assay of crude tissue extracts initially revealed the widespread occurrence of PKC activity, with brain tissue being a rich source of the enzyme (103, 125, 156, 111). An uneven pattern of expression in brain tissue was first documented by measuring the autoradiographic distribution of [^{3}H]PDBu binding in calf and mouse brain (161, 162), and also in rat brain (249–252). Following the discovery of multiple PKC subspecies by cDNA sequence analysis, in situ hybridization studies, using probes complementary to the individual cDNA sequences, revealed that the PKC transcripts exhibit a different regional and cellular distribution in brain and other tissues (119, 30, 175, 184). Direct biochemical analysis combined with immunoblotting techniques has confirmed this qualitative difference and also revealed a quantitatively different level of expression of the α/βI/βII/γ-group of enzyme subspecies in various regions of the brain and other tissues (212–214, 96, 97, 120). Immunocytochemical studies with polyclonal antisera raised against a purified, unresolved mixture of PKC subspecies (74, 247, 94), and with subspecies-selective monoclonal and polyclonal antibodies (117, 97, 99, 83, 204, 256, 84, 9, 121, 262, 263) has revealed that the α/βI/βII/γ-group of enzymes is differentially expressed at the cellular and subcellular level. Most cell types so far studied coexpress more than one subspecies of PKC, although A 431 (101), COS 7 (181), NIH 3T3 (151), 3T3-L1 (97), and Swiss 3T3 cells (200) contain only the α-subspecies, whereas rat ovary granulosa cells (97) have been reported to contain only the β-subspecies. A detailed description of the rapidly accumulating body of information concerning these distribution patterns is beyond the scope of this article, but can be obtained by consulting the above references. Table 1 gives a brief summary of the available information (see also 171).

The expression of PKC subspecies described above can be considered as that occurring in the unstimulated state of the cells. There is evidence, however, that the level of expression of PKC subspecies can change during development (83) or cell differentiation (142), or can be altered by certain stimuli. Exposure of cells to agents that activate PKC in a persistent manner, such as TPA, for example, can promote the proteolytic degradation of the enzyme. It appears that it is the active conformation of PKC that is susceptible to this. The rate of resynthesis of the enzyme is insufficient to compensate for this acute loss, so that a sustained disappearance of PKC activity from the cell may occur (257). Recent work from this laboratory has shown that multiple subspecies of PKC coexpressed in a single cell type can disappear at different rates upon treatment with TPA (10), and that this

§ It should be noted that this profile definition only applies to the papers from the laboratories in which it was originally described. Recent studies from a number of other laboratories, using tissue other than brain, or using hydroxyapatite gel from different sources, have reported the resolution of multiple fractions of phospholipid- and Ca^{2+}-dependent protein kinase activity, often designated type I, II, III, and so on. In most cases, however, the correspondence of these fractions with the brain γ, β, and α PKC subspecies has not been established adequately. The order of elution from the column is not, in itself, a sufficient basis to allow this.

may be due to their relative susceptibility to the action of calpain I (116). If such a degradatory mechanism is initiated during prolonged activation of PKC as a result of a biological stimulus, then the relative expression of the PKC subspecies would be perturbed. This could have profound effects on the modulation of the cellular response to subsequent stimuli.

B. General Properties of Ion Channels

1. Structure. Molecular cloning analysis has provided important data concerning the primary sequence of several ion channel proteins, the comparison of which has shown them to belong to a related molecular family, in an analogous fashion to the receptor 'superfamilies.' Hydropathy profiles of the sequences available at present reveal the presence of conserved structural domains, and suggest that 'voltage-operated' [such as sodium (Na^+), potassium (K^+), and Ca^{2+}] and 'ligand-operated' (such as the nicotinic acetylcholine (nACh) receptor-associated and the γ-aminobutyric acid $(GABA)_A$ receptor-associated) channels fall into subgroups within the structural family. The two groups differ in the polypeptide composition of the channel, i.e., multiple subunits versus a single, large principal polypeptide, and in the arrangement of their putative transmembrane domains. They appear, however, to utilize a similar pseudosymmetrical array of homologous transmembrane α-helical segments to form a ring structure that serves as the channel pore (140, 39). Point mutation analysis of negatively charged and polar amino acids between the proposed M1, M2, and M3 transmembrane segments of the *Torpedo* nACh receptor subunits has indicated that in this case, the net negative charge of the side chains is the major determinant of the rate of ion transport through the channel (102). The presence of rings of charged amino acid residues at equivalent positions in other multisubunit ligand-operated channels, such as the $GABA_A$ receptor (207), suggest that this may be a common structure:activity motif.

2. Biophysical Properties. It is now clear that ion channel proteins function as dynamic entities, and are of fundamental importance for the maintenance, and when necessary, the dissipation of ionic gradients in both excitable and nonexcitable cell types. The net direction of currents through ion channels is determined principally by the electrochemical gradient of the ions across the membrane in which the channel resides, although this movement is counterbalanced by the membrane potential. Generally, the process is electrogenic, so that it is possible to measure it as a membrane current when the voltage across the membrane is held constant. Recent improvements in methodology, instrumentation, and the availability of pharmacological agents have greatly facilitated studies on the biophysical properties of ion channels, and how they may be affected. In particular, the use of the patch clamp technique, as popularized by Sigworth and colleagues (79), to study conductance properties at the single channel level, has revolutionalized the field of electrophysiology in a manner akin to the effect of the ligand binding technique on receptor pharmacology. Using this and other techniques, ion channels have now been shown to exist as a diverse family, with numerous pharmacologically distinct subtypes each possessing unique conductance properties. This ubiquity and diversity at the molecular level is exploited at the cellular level, where they participate in a wide variety of cellular processes, such as neuronal excitability, excitation-contraction coupling, and secretion.

3. Modulation. The innate properties of ion channels arise from their ability to adopt more than one functional conformation, and the fact that the rapid transition between these conformational states can be regulated by a number of different factors. Conductance of ions through the channel pore may be altered by the presence of competing ions, or by other molecules such as Ca^{2+} and 1, 4, 5-IP_3, which in some instances (section IIA; see also 87), seem to act directly on certain types of ion channels, in addition to their role as intracellular messengers. Channel activity may also be modified by interaction of the channel molecule with a G-protein. The direct interaction of ion channels with G-proteins is a recent, but not surprising, refinement to the consideration of ion channel modulation at the macromolecular level. Many voltage-operated K^+ and Ca^{2+} channels have been shown already to be regulated by them (see 236 and 91).

Direct phosphorylation of ion channel proteins (or possibly phosphorylation of one of the subunit components that interacts with the principal channel protein, and thereby affects its intrinsic properties), can bring about long term changes in their activity (129, 107, 108, 198, 201). Covalent modification feasibly may alter one of a number of parameters, such as the open probability of the channel, its unitary conductance (although an example of this has yet to be described), or the number of functional channels within the membrane. In many cases, for example, the serotonin-sensitive 'S-channel' of *Aplysia* sensory neurones (carrying a voltage-operated K^+ current), the channel protein can serve as a convergent substrate for more than one protein kinase enzyme (21). The action of each kinase may result in a similar change in the channel current amplitude, or have the opposite effect, and could be achieved by affecting a different one of these parameters.

II. Modulation of Ion Channel Activity by Protein Kinase C

All of the work to be described below has appeared in the literature within the last 5 years. This fact attests to the rapid development and application of the methodology for studying ion channel currents in a wide range of tissues and cells, and the intense interest in the role of

PKC in modulating this process. The information has been separated into sections on the modulation of Ca^{2+} channels, K^+ channels, and 'others.' Each section is further divided into subsections on the various kinds of tissues and cells in which the modulation of the channels has been investigated.

A. Calcium Channels

The experimental findings describing the modulation of Ca^{2+} channel activity by PKC are summarized in table 2.

1. Channel classification.‖ Alteration of the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, is a crucial step in the regulation of a variety of cellular processes such as contraction, secretion, energy metabolism, neurite outgrowth/retraction, and gene transcription. Changes in $[Ca^{2+}]_i$ can be elicited physiologically by mobilizing Ca^{2+} from intracellular storage pools and/or by increasing the Ca^{2+} permeability of the plasma membrane (reviewed in 153). Influx of Ca^{2+} from the extracellular medium is known to occur either through ligand-operated or voltage-operated Ca^{2+} channels of the plasma membrane. Ca^{2+}-permeable ligand-operated channels can be further divided into two classes: receptor-operated channels, such as the ATP-activated Ca^{2+} channels in rabbit ear artery smooth muscle cells (22), and those recorded in membrane vesicles from thrombin-activated blood platelets (260), and second-messenger operated channels, such as those altered by Ca^{2+} (in neutrophils, 232) and 1, 4, 5-IP_3 (in T-lymphocytes, 124; in mast cells, 190). Voltage-operated Ca^{2+} channels are known to be quite heterogenous in nature, with the existence of multiple types of Ca^{2+} currents having been shown in numerous neuronal and nonneuronal cells by voltage-clamp methods. Single channels recordings in chick dorsal root ganglion (DRG) neurones have identified a low threshold voltage-operated channel [called the T (transient)-type, $I_{Ca(T)}$] and two kinds of high threshold voltage-operated channels [called L (long-lasting)-, $I_{Ca(L)}$, and N (neuronal)-types, $I_{Ca(N)}$] (37, 172, 65, 253, 69, 70). The three types of channels can be distinguished on the basis of their gating properties, ion conductance, and pharmacology. Their basic properties will be summarized here, but readers are referred to several recent articles (155, 88, 233, 91) for a more detailed discussion. T-type channels generally activate at relatively negative potentials, carry small unitary currents (approximately 8 pS slope conductance, with barium (Ba^{2+}) ions as the carrier), and produce a rapid, transient, average current. The channels are blocked only weakly, or not at all, by ω-conotoxin GVIA (ω-CgTx; 199, 152), and are resistant to 1,4-dihydropyridine (DHP) derivatives. T-type channels from various cells exhibit very similar properties. L-type channels are activated at less negative potentials, carry large unitary Ba^{2+} currents (approximately 25 pS slope conductance), and generate a long-lasting average current. They are opened selectively by the DHP agonist (-)BAY K 8644 (as the L-type channel is the only type of Ca^{2+} channel so far identified that is sensitive to DHPs, it is also referred to as the 'DHP-receptor'). They are antagonized by the DHP derivatives nitrendipine and nifedipine, and in certain neurones (but not muscle cells), ω-CgTx reduces $I_{Ca(L)}$ (152). Two other distinct classes of organic Ca^{2+} channel antagonists, the phenylalkylamines (such as verapamil), and the benzothiazepines (such as diltiazem) act at separate, allosterically linked receptor sites on the L-type channels. These properties are fairly conserved among the L-type channels found in many tissues, although the voltage dependency can be variable. It is likely that neuronal and smooth muscle L-type channels form a further subgroup. N-type channels activate at negative holding potentials and carry Ba^{2+} currents of intermediate magnitude (approximately 13 pS slope conductance). So far, they have been reported to exist only in neuronal cells, where they may participate in neurotransmitter release (155). They are insensitive to DHPs, but can be blocked by ω-CgTx.

The DHP-sensitive L-type channel from skeletal muscle has been purified by conventional methods, and found to be a complex of five polypeptides (39 and references therein). The polypeptide α_1 [M_r 165(175)k] expresses the binding sites for the three classes of antagonist mentioned above, and has extensive hydrophobic domains. By analogy with the α-subunit of the voltage-operated Na^+ channel, it is thought to be the principal functional component of L-type channels, and to form the channel pore. Associated with the α_1-subunit isolated from skeletal muscle are a variable number of polypeptides [α_2, 143(170)kDa; β, 54kDa; γ, 30kDa; and δ, 27kDa].

The precise function of these subunits is not known at present, although the appropriate cyclic AMP-dependent protein kinase (protein kinase A, PKA)-mediated phosphorylation of the α_1-and β-subunits, seems to be essential for the functional activity of the purified, reconstituted channel (67, 39). It would seem likely that T- and N-type channels will be found to possess a very similar structure to the L-type, but (minimally) with altered amino acid sequences around the regions of the α_1 subunit responsible for binding the three classes of Ca^{2+} channel antagonist.

2. Invertebrate cells. Exogenous application of PKC activators, or intracellular injection of a partially purified, unresolved mixture of PKC subspecies from rat brain, into *Hermissenda* Type B photoreceptors, pro-

‖ There is not, as yet, a uniform system of written nomenclature for the numerous species of ion channels and the currents that they carry. In this article, for simplicity, we identify the current firstly by the principal ion that it carries, and, secondly, in parentheses, by a commonly used suffix. This suffix may indicate a molecule that regulates the channel, and/or a subclassification of the channel within similar species of channel.

TABLE 2

Modulation of calcium channel activity by protein kinase C activation

Cell type	Recording method*	Stimulus	Current response	Reference
Hermissenda B photoreceptors	*a*	PDBu (1–10 nM) PKC injection	↑ I_{Ca}	63
	a	OAG (2.4–120 μM) DPBA (1–100 nM)	→ I_{Ca}	5
Aplysia bag cell neurones	*a, b*	TPA (1–200 nM) DiC_8 (3–15 μM) PKC injection	↑ I_{Ca}	52, 226
Helix aspera neurones	*a*	CCK8 (5 nM) OAG (250 nM, 60 μM) TPA (400 pM) PKC injection	↓ I_{Ca}	80
Rat hippocampal CA1 pyramidal neurones	*a*	PDBu (10 μM)	↑ I_{Ca}	14, 144
Guinea-pig and rat fetal hippocampal pyramidal neurones	*a*	PDA (1.5 μM) PDBu (100 nM–2 μM) OAG (60 μM)	↓ $I_{Ca(N)}$ ↓ $I_{Ca(L)}$ → $I_{Ca(T)}$	54
Rat dorsal raphe neurones	*a*	PDBu (10–100 nM) PDA (100–500 nM)	↓ $I_{Ca(T)}$	71, 72
Xenopus oocyte expression system (rat brain mRNA)	*a*	PDBu (100 nM) OAG (100 μM)	↑ I_{Ca}	128
Xenopus oocyte expression system (chick brain mRNA)	*a*	TPA (10 nM) OAG Quisqualate (10 μM)	↑ I_{Ca}	219
Chick dorsal root ganglion neurones	*a*	OAG (600 nM–60 μM) DPBA (10 nM–50 μM)	↓ $I_{Ca(L)}$	194
Chick embryo dorsal root ganglion neurones	*a*	OAG (40 μM)	↓ $I_{Ca(L)}$ ↓ $I_{Ca(T)}$	149
Rat neonatal dorsal root ganglion neurones	*a*	TPA (100 nM)	↓ $I_{Ca(T)}$	208
Mouse dorsal root ganglion and cerebral hemisphere neurones	*a*	TPA (1–100 nM) PDBu (10 nM–1 μM)	↓ $I_{Ca(N)}$ → $I_{Ca(L)}$ → $I_{Ca(T)}$	243, 244, 77
Chick embryo sympathetic ganglion neurones	*d*	PDBu (3–10 nM) TPA (3–10 nM)	↑ I_{Ca}	147
NG108-15 neuroblastoma × glioma cell line	*c*	OAG (100 μM) TPA (100 nM–1 μM)	↑ I_{Ca}	186
	a	PDBu (10 nM–1 μM) OAG (100 nM–10 μM)	→ I_{Ca}	35
PC 12 pheochromocytoma and RINm5F insulin-secreting B-cell line	*c, d*	TPA (1–100 nM) OAG (50 μM)	↓ $I_{Ca(L)}$	53
PC 12	*d*	TPA (600 pM–600 nM)	↓ $I_{Ca(L)}$	82
	d	TPA (10 nM–10 μM) PDBu, PDA	↓ $I_{Ca(L)}$	154
Chick embryo skeletal muscle myotubes	*d*	TPA (1–100 nM)	↑ $I_{Ca(L)}$	165
Chick embryo cardiac ventricular myocytes	*c, d*	TPA (10 nM–1 μM)	↓ I_{Ca}	129
Rat neonatal cardiac ventricular myocytes	*a*	TPA (10–85 nM) Angiotensin II	↑ $I_{Ca(L)}$	56
	b, d	TPA (10 nM–1 μM) DiC_8 (2 μM) TPA (10 nM–1 μM)	↑ $I_{Ca(L)}$ ↓ $I_{Ca(L)}$	126
Guinea pig cardiac ventricular myocytes	*a*	TPA (1 nM)	→ I_{Ca}	231
Rat cardiac ventricular myocytes	*a*	TPA (10 nM–1 μM) OAG (60 μM) Phenylephrine (5 μM) Methoxamine (5–10 μM)	→ I_{Ca}	8
Toad gastric smooth muscle cells	*a*	DiC_8 (60 μM) Acetylcholine (50 μM)	↑ $I_{Ca(L)}$ → $I_{Ca(T)}$	239
Porcine coronary artery smooth muscle	*c*	TPA (100 nM)	→ I_{Ca}	105

TABLE 2
Continued

Cell type	Recording method*	Stimulus	Current response	Reference
Rabbit aorta smooth muscle	*d*	PDBu (1–10 μM)	→ I_{Ca}	227
Rabbit thoracic aorta smooth muscle	*d*	PDBu (100 nM–10 μM)	↑ I_{Ca}	75
Dog saphenous vein smooth muscle	*d*	PDBu (1 μM)	↑ $I_{Ca(L)}$	41
Rat aorta smooth muscle	*d*	PDBu (100 nM–1 μM)	↑ $I_{Ca(L)}$	133
A_7r_5 vascular smooth muscle cell line	*a, d*	TPA (1–100 nM) PDBu (100 nM)	↑ $I_{Ca(L)}$	223, 66
	a, d	PDBu (30 nM–10 μM) TPA (3 nM–1 μM) OAG (100 μM) DiC_8 (100 μM) Vasopressin (100 nM)	↓ $I_{Ca(L)}$	73, 237
GH_4C_1 rat pituitary cell line	*c*	OAG (50 μM) DiC_8 (29 μM) TPA (200 nM)	↑ $I_{Ca(L)}$	4
Rat anterior pituitary gonadotrophes	*c*	TPA (1 μM)	↑ $I_{Ca(L)}$	211
GH_3 rat pituitary cell line	*a*	OAG (4–60 μM)	↓ $I_{Ca(L)}$ ↓ $I_{Ca(T)}$	149
AtT-20 mouse pituitary cell line	*a*	OAG (10–100 μM) DPBA (10 μM) PDA (100 μM) TPA (10–100 nM)	↓ $I_{Ca(L?)}$	130, 135
Rat anterior pituitary prisms	*d*	TPA (100 nM) OAG (63 μM)	↓ I_{Ca}	157
Rat adrenal medulla	*d*	PDBu (30 nM)	↑ I_{Ca}	240
Bovine adrenal chromaffin cells	*c*	TPA (160 nM)	↓ $I_{Ca(L)}$	193
Mouse 3T3 and human fibroblasts	*b*	TPA OAG	↑ $I_{Ca(L)}$	40
UMR-106 osteosarcoma cell line	*c*	TPA (2 μM) OAG (500 μM) PDA	↑ $I_{Ca(L?)}$	255

* Recording methods: *a*, whole cell recording under current- or voltage-clamp configuration; *b*, cell-attached membrane patch voltage-clamp; *c*, organic calcium ion indicator; *d*, ion flux measurements. Abbreviations and channel nomenclature as given in the text.

duced a rapid, persistent enhancement of the steady state depolarizing response to light, mimicking the changes caused by associative training in this animal (63, 5). Several different ionic currents appear to underlie these changes. Under voltage-clamp conditions, one of the currents involved, I_{Ca} (not defined further), carried by a voltage-operated Ca^{2+} channel, was found either to be enhanced (63) or unchanged (5) following PKC activation. Under the same conditions, two species of K^+ current were reduced (table 3).

As described for *Hermissenda* Type B photoreceptors, exposure of *Aplysia* bag cell neurones to TPA, the synthetic DAG 1,2-dioctanoylglycerol (DiC_8) or intracellular injection of a mixture of PKC subspecies from bovine brain, leads to an increase in the voltage-operated Ca^{2+} current, I_{Ca}, measured with Ba^{2+} ions in the recording pipette (52, 226). In this case, however, the increase in inward current following PKC activation was due to the recruitment of a uniformly distributed, previously covert class of channels with a higher unitary conductance (approximately 24pS), while current through the smaller channel (slope conductance approximately 12pS), was unaffected by TPA treatment. This may be a mechanism by which prolonged increases in the capacity for Ca^{2+} influx into the cell is mediated. In *Aplysia* bag cell neurones, activation of PKC or PKA result in the same net effect, i.e., enhancement of the action potential amplitude, without changes in its duration or the afterhyperpolarization (AHP), but do so by different mechanisms. PKC stimulates inward Ca^{2+} currents, without directly affecting outward K^+ currents, whereas PKA inhibits outward K^+ currents (see 129, and section II B), without directly affecting voltage-operated Ca^{2+} currents.

In contrast to the results in *Hermissenda* and *Aplysia*, application of cholecystokinin octapeptide 26–33 (CCK-8), activators of PKC, or injection of a mixture of PKC subspecies purified from bovine brain into the identified snail neurones D2 and F77 from *Helix aspera*, was found to cause a persistent reduction of the peak inward Ca^{2+} current, resulting in a shortening of the action potential duration (80). The PKC-mediated effect required the presence of cytosolic Ca^{2+} but was not mimicked by the injection of 1, 4, 5-IP_3. PKA and cyclic GMP-dependent protein kinase (protein kinase G, PKG) also were without effect.

3. Vertebrate central nervous system neurones. In rat hippocampal CA1 pyramidal cells, the predominant effect of PKC activation is to reduce the amplitude of a Ca^{2+}-activated K^+ current, $I_{K(Ca(S))}$, that underlies the slow AHP (see section II B, table 3). However, under recording conditions where the voltage-operated Na^+ and K^+ currents were blocked [by the presence of tetrodotoxin and tetraethylammonium chloride (TEA), respectively], phorbol esters were also found to increase slightly the calcium spike amplitude, suggesting an enhancement of I_{Ca} (14, 144). On the other hand, in preparations of acutely dissociated guinea pig hippocampal cells, and cultured fetal rat hippocampal cells, whole cell voltage-clamp analysis showed that various PKC activators markedly reduced, in a reversible manner, $I_{Ca(N)}$, with less effect on $I_{Ca(L)}$ and no effect on $I_{Ca(T)}$ (54). These effects could be antagonized by H-7 and polymixin B, but not by the Ca^{2+}/calmodulin antagonist calmidazolium. 8-Bromo-cAMP was also without effect. In this cell preparation, voltage-operated K^+ currents were also found to be suppressed, in agreement with previous reports (table 3). A similar PKC-mediated selective reduction of $I_{Ca(N)}$, and certain K^+ currents, has also been reported in mouse cerebral hemisphere neurones (243, 244), and two brief reports have also indicated that phorbol esters may suppress the firing of rat dorsal raphe neurones by inhibiting low threshold Ca^{2+} currents ($I_{Ca(T)}$?; 71, 72). These papers represent a clear indication of a postsynaptic site of action of PKC in central nervous system neurones.

Total messenger RNA (mRNA) isolated from rat brain (128) and chick brain (219) has been injected into *Xenopus* oocytes, leading to the expression of functional ion channels of several different classes. From the rat brain transcripts, Ba^{2+} currents through the expressed Ca^{2+} channels, studied under voltage clamp conditions, were found to be enhanced by treatment with PDBu or 1-oleoyl 2-acetylglycerol (OAG), but not by forskolin (128). Using chick brain mRNA, I_{Ca} was also found to be enhanced by active phorbol esters, and inhibited by tamoxifen (219). These effects could be mimicked by the glutaminergic agonist, quisqualate. PKC activation was also found to influence the current through a number of other channels (section II C, table 4). This technique represents a potentially very fruitful approach to the study of ion channel modulation at the molecular level. However, the use of total mRNAs leads to the expression, in the same cell, of many types of receptors and regulatory proteins, as well as ion channels. Although this may present opportunities for studying novel interactions between transduction systems, it may also provide misleading observations. A modified approach, for example using size fractionation of the mRNA transcripts to limit the expression of proteins, coupled with single channel recording, could prove to be a very powerful technique.

4. Vertebrate peripheral neurones. In peripheral neurones, many inhibitory agents reduce the duration of the action potential, resulting in a decrease in neurotransmitter release. This may be due either to an enhancement of I_K or to an inhibition of I_{Ca}. DRG sensory neurones possess all three types of voltage-operated Ca^{2+} channels, as well as a number of different types of K^+ channel. In embryonic chick DRG cells held at a potential at which $I_{Ca(T)}$ and $I_{Ca(N)}$ were largely inactive, OAG and 12-deoxyphorbol 13-isobutyrate 20-acetate (DPBA) were found to produce a rapid, reversible decrease in the peak amplitude of $I_{Ca(L)}$, without affecting voltage-operated K^+ currents (194). This observation has been confirmed in chick DRG cells by Marchetti & Brown (149), who, by using a depolarizing voltage pulse protocol that allowed the separation of the $I_{Ca(T)}$ and $I_{Ca(L)}$ components of the whole cell Ca^{2+} current, showed that $I_{Ca(T)}$ also was decreased reversibly by OAG-promoted PKC activation in these neurones. In a brief communication, Schroeder et al. (208) have reported that prolonged exposure to TPA, but not acute exposure, results in a decrease in the proportion of cultured rat neonatal DRG cells expressing $I_{Ca(T)}$. In primary cultures of mouse DRG neurones, on the other hand, phorbol esters have been reported to selectively reduce $I_{Ca(N)}$, in a reversible manner, without affecting either $I_{Ca(T)}$ or $I_{Ca(L)}$, as well as reducing K^+ currents (243, 244, 77; section II B, table 3). Selective reduction of $I_{Ca(N)}$ could also be produced by application of either forskolin (100 μM) or 8-bromo-cAMP (1 mM), implying that this channel was a common target for both PKC and PKA. The action of phorbol esters was prevented by pretreatment of the cells with *Pertussis* toxin, whereas that by forskolin or 8-bromo-cAMP was not (77; compare the effect in AtT-20 pituitary cells). It is possible that, in this case, PKC exerts its negative action on I_{Ca} by inhibiting the inhibitory G-protein, G_i, and thereby raising cAMP levels.

An important finding concerning the modulation of ion channel activity in DRG neurones, is that chronic phorbol ester treatment leads to the down-regulation of PKC from these cells. Following such treatment, the ability of neuropeptide Y (NPY) to inhibit the sustained portion of the Ca^{2+} current in rat DRG cells (mediated by $I_{Ca(N)}$) was found to be attenuated greatly, whereas the transient portion (mediated by $I_{Ca(T)}$ and $I_{Ca(N)}$ still could be reduced substantially (61; see also section on PC 12 cells). This is a good indication that PKC is involved at some stage of the NPY inhibition of $I_{Ca(L)}$ (and also that a further action of the transmitter involving another intracellular mechanism is needed for the modulation of $I_{Ca(T)}$ and $I_{Ca(N)}$). The receptors for the inhibitory neurotransmitters on these cells, such as NPY, noradrenaline and GABA ($GABA_B$), are known to be linked to G-proteins, as their effects can be blocked by GDP-β-S, a nonhydrolyzable analogue of GDP, or by *Pertussis* toxin treatment, and mimicked by intracellular administration of GTP-β-S (89, 55, 241). In the case of

noradrenaline and GABA, the receptor-mediated inhibition of I_{Ca} seems to involve a G-protein directly (89), but this interaction is regulated in a complex manner. The effect of GABA can be mimicked by direct activation of PKC, and it has been proposed that this may be the result of production of arachidonic acid via another *Pertussis* toxin-sensitive G-protein-linked PLA_2, rather than by phospholipase C-catalyzed production of DAG (29). Clearly, aside from the discrepancies in experimental observations between species, in DRG neurones the modulation of Ca^{2+} function takes place at different levels of the signal transduction pathway, with G-proteins, that apparently interact with both catalytic enzymes and channel proteins, being the site of convergent modulation by more than one kinase system.

Interestingly, in hippocampal pyramidal cells, the slow hyperpolarizing response evoked by GABA or the $GABA_B$ receptor agonist, baclofen, is the result of an increase in a Ca^{2+}-independent K^+ current, rather than inhibition of I_{Ca} (7). In both cell types, a *Pertussis* toxin-sensitive G-protein couples the receptor to the ion channel; this is a common arrangement in hippocampal cells also shared by the serotonin 5-HT_{1A} and adenosine A_1 receptors (166). In contrast to the situation in DRG cells, though, the $GABA_B$-mediated response in hippocampal cells is blocked by activation of PKC. Therefore, although the receptor and G-protein are apparently the same in both cases, the net effect on the ion channel, and the membrane current is the opposite.

In contrast to DRG neurones, in cultured chick embryo sympathetic ganglion neurones, phorbol ester activation of endogenous PKC activity was found to be associated with an enhanced electrically stimulated accumulation of $^{45}Ca^{2+}$ and resultant release of transmitter (147). In addition, in this particular neuronal population, PDBu also caused an increase in the basal accumulation of $^{45}Ca^{2+}$ and the spontaneous release of transmitter, an effect rarely reported.

5. NG108–15 neuroblastoma x glioma hybrid cell line. Two types of Ca^{2+} current have been reported in NG108–15 hybrid cells, resembling $I_{Ca(T)}$ and $I_{Ca(L)}$. OAG and TPA were reported initially to stimulate Ca^{2+} influx through voltage-operated Ca^{2+} channels, and to inhibit the response to bradykinin in these cells (186, 187). Recent detailed electrophysiological analysis of the effects of bradykinin, Ca^{2+}, 1, 4, 5-IP_3, and PKC activators on channel currents has concluded, however, that the net inward current produced by PKC activation is a result of inhibition of the outward 'M-current,' $I_{K(M)}$ (a time- and voltage-operated K^+ current; section II B, table 3), and not a stimulatory action on I_{Ca}, the latter being a consequence of the former (35, 36).

6. PC 12 neurosecretory cells. In the rat pheochromocytoma PC 12 neurosecretory cell line, phorbol ester treatment results in the rapid, concentration-dependent decrease in depolarization- or DHP agonist-evoked Ca^{2+} influx through L-type channels, without a concomitant effect on Ca^{2+} efflux, to give a decrease in $[Ca^{2+}]_i$ (53, 82, 154). TPA treatment has been reported also to reduce the depolarization-induced $[Ca^{2+}]_i$ rise in the insulin-secreting pancreatic B-cell line, RINm5F (53; but see 246, table 3). It would seem that PKC only modulates the activated form of the L-type channel, as it has little effect under nondepolarizing conditions.

In the case of PC 12 cells, this action of PKC appears to be separate from an additional role in the secretory process, as treatment of PC 12 cells with phorbol esters leads to an enhancement of depolarization-induced [^{3}H]dopamine and [^{3}H]noradrenaline release (192, 82). Prior down-regulation of PKC by chronic phorbol ester treatment was shown to block subsequent phorbol ester enhancement of depolarization-induced transmitter release and to attenuate depolarization-induced release itself (150). The remaining depolarization-induced transmitter release in PKC-depleted cells could be inhibited by a DHP antagonist, implying that the inhibition of L-type channels had been relieved in these cells. This PKC-independent mechanism is presumably mediated by another Ca^{2+}-dependent kinase system.

7. Skeletal and cardiac muscle cells. In skeletal muscle, contractile force is not dependent on Ca^{2+} influx, as in cardiac and smooth muscle, but on Ca^{2+} release from the sarcoplasmic reticulum. DHP receptors are present at a high density in the transverse tubule system, although the correlation between the number of DHP binding sites and the number of functional L-type channels is not known with any certainty. It has been proposed that in skeletal muscle, the DHP receptor molecules may act as voltage sensors (i.e., a protein containing a group of membrane spanning amino acids with dipole properties, that react to changes in the electric field by reorientation), involved in the stimulation of Ca^{2+} release, as well as components of voltage-operated Ca^{2+} channels (228). Further support for this proposal was provided recently, with the finding that in cultured muscle cells from mutant mice with muscular dysgenesis, microinjection of a plasmid that permitted the expression of the cloned α_1-subunit (DHP receptor) gene restored the slow Ca^{2+} current, as well as excitation-contraction coupling (229). Early work demonstrated that skeletal and cardiac muscle Ca^{2+} currents could be regulated by cAMP (185, 197, 20). The 165(175)kDa α_1-subunit, which constitutes the principal channel polypeptide, can be phosphorylated by PKA (49, 90), and in the reconstituted channel this causes a prolongation of the open probability, suggesting that direct phosphorylation regulates the function of L-type channels in skeletal muscle cells (67). Phosphorylation of the α_1-subunit has also been reported using the multifunctional Ca^{2+}/calmodulin kinase (90) and a purified mixture of brain PKC subspecies (164, 173). In the case of PKC, there is no electrophysiological data with skeletal muscle cells on the modulation of $I_{Ca(L)}$, but in

primary cultures of chick embryo pectoralis muscle, TPA or OAG treatment has been found to enhance DHP-sensitive $^{45}Ca^{2+}$ uptake (165). This latter report is interesting, as the treatment was associated with an increase in the number of specific binding sites for the DHP-antagonist (+)[^{3}H]PN200–110. cAMP was shown previously to increase the number of DHP receptors in the same cell type, apparently by regulating their synthesis (206). The effect of TPA, however, was not changed by the presence of cycloheximide, implying that de novo protein synthesis was not involved. This situation resembles that described above in *Aplysia* bag cell neurones (52, 226). It will be of interest to learn whether or not the increased number of DHP-receptor sites is a result of the active recruitment of additional channels from a subcellular compartment (and if these are of the same class as those present before PKC activation), or merely an unmasking of binding sites on channel subunits already present in the plasma membrane. The similarities between the biochemical actions of PKA and PKC suggest that skeletal muscle L-type channels may be a direct target of PKC. The functional consequences of phosphorylation of the α_1-and β-subunits (see 164), and the relative importance of each one, however, remains to be determined.

In myocardium, contractile force is directly related to Ca^{2+} influx. Although an enzymatic activity of PKC has been demonstrated biochemically (245), and the resolution of PKC subspecies achieved (120), its physiological role in this tissue has not been elucidated. Leatherman et al. (127) found that TPA caused a time- and concentration-dependent decrease in the contraction amplitude (inotropic response) of cultured chick embryo ventricular myocytes, associated specifically with a decrease in Ca^{2+} influx. A decrease in the contraction amplitude, that was concurrent with an increased beating rate (chronotropic response), was observed also in TPA-treated rat neonatal ventricular myocytes by Dosemeci et al. (56). This effect was mimicked by angiotensin II (known to increase PIP_2 turnover), and both stimuli were found to increase the endogenous phosphorylation of the same set of proteins. In complete contrast to the report of Leatherman and colleagues, however, voltage-clamp analysis revealed a PKC-mediated increase in both the transient and steady state components of the cadmium-sensitive $I_{Ca(L)}$. Clearly, this observation is not readily explained in terms of the negative inotropic response. Lacerda et al. (126), using ion flux measurements and single channel recording techniques, have made the observation that TPA has a biphasic effect on cardiac L-type channels. Short-term (5 s) exposure to TPA resulted in an increased open probability of the channels, that was mimicked by DiC_8, but not by 4-α-phorbol. Longer exposure (20 min) to TPA, however, significantly reduced conductance through the channel. This inhibitory effect was not mimicked by DiC_8, which continued to be stimulatory, which has prompted these authors to suggest that a perturbation of the PKC levels may underlie the inhibitory effect of TPA. A relatively straightforward experiment to measure PKC activity following this treatment should clarify this. Support for these observations is provided also by an earlier report of Teutsch et al. (230). TPA was found to have a dual action on the inotropic response of electrically stimulated guinea pig left atria: an initial enhancement followed by a decline in contractile force, whereas DiC_8 produced only a positive effect.

As discussed in section II B, elucidation of the role of PKC in the contractile responses of myocytes to phorbol esters has been approached from the point of view of K^+ channel modulation. While reporting such studies, some groups have found no change in the whole cell Ca^{2+} current following the application of TPA, OAG, or α_1-adrenergic agonists (231, 8; section II B, table 3). The wide variation in experimental observations described above, and also discussed later, indicates that although an action of PKC at some level of the contractile process is likely, the exact nature of this remains controversial.

8. Smooth muscle cells. In freshly dissociated toad gastric smooth muscle cells, both $I_{Ca(L)}$ and $I_{Ca(T)}$ currents have been observed using whole cell recording techniques (239). DiC_8 and acetylcholine both selectively enhance $I_{Ca(L)}$ in these cells, whereas the structurally similar compounds DiC_8-SH and DiC_8-Cl, which are not effective as activators of PKC, do not (239). These observations suggest that DAG is likely to be the second messenger responsible for the muscarinic effect on $I_{Ca(L)}$ in visceral smooth muscle cells.

In vascular smooth muscle, a number of agonists, such as serotonin, acetylcholine, and noradrenaline, have been shown to increase Ca^{2+} flux in association with increased contractile activity. Neither agonists acting at the β-adrenoceptor nor cAMP analogues appear to have direct effects on Ca^{2+} entry into smooth muscle, although they may reduce $[Ca^{2+}]_i$ by promoting sequestration, and so, modification of Ca^{2+} channel activity by PKC might be considered as a plausible mechanism for controlling Ca^{2+} entry. Several different groups have investigated this possibility, but have arrived at conflicting results. The contractile responses to phorbol esters reported using rat and rabbit aorta (50), dog basilar artery (15), and dog saphenous vein (41) were found to be dependent, to varying degrees, on extracellular Ca^{2+}. In rabbit ear artery (68) and rat aorta (133), the response was markedly enhanced by raising $[Ca^{2+}]_i$ using an ionophore or (−)BAY K 8644, and in rabbit aorta (227, 75), or rat aorta (222), it was found essentially to be independent of extracellular Ca^{2+}. Direct measurement of the effects of phorbol esters on Ca^{2+} influx have also produced variable responses (table 2). Using either quin 2 fluorescence or $^{45}Ca^{2+}$ influx as an index of Ca^{2+} entry, phorbol esters have been reported to cause no change in Ca^{2+} influx (227, 105), to stimulate Ca^{2+} influx (75, 41) or to

potentiate Ca^{2+} entry in cells treated with (−)BAY K 8644 (133).

The spontaneously active A_7r_5 clonal vascular smooth muscle cell line also possesses L- and T-type channels (66). Ca^{2+} flux measurements (223) and whole cell voltage-clamp analysis (66), showed that active phorbol esters increased the $I_{Ca(L)}$. In the former study, however, this treatment was found not to increase $[Ca^{2+}]_i$, as Ca^{2+} efflux was also stimulated. In contrast to these results, work by another laboratory has shown that various activators of PKC, and agonists that stimulate PIP_2 breakdown, cause an inhibition of L-type channel activity (73, 237). More extensive voltage clamp analysis of the effects of [Arg^8]vasopressin, suggest, however, that the inhibitory action of these agents is not straightforward. Inhibition of $I_{Ca(L)}$ could be affected by an increase in $[Ca^{2+}]_i$ induced by A23187, by the injection of 1, 4, 5-IP_3, as well as by PKC activation (237).

The discrepancy between the experimental results described above is not readily explainable. The relative contribution of intracellularly and extracellularly derived Ca^{2+} to the activation of the Ca^{2+}/calmodulin-dependent myosin light chain kinase, and the subsequent contractile response, and the modulatory role (if any) of PKC, in this and the sustained phase of the contraction, is still unclear. There appears to be a tissue-dependent factor involved (which may be exacerbated by the differences in methodology employed), that may reflect the differential distribution of multiple PKC subspecies (there is a paucity of information on this), or the heterogeneity of L-type channels. The use of single-channel recording techniques should clarify the latter point, and also give valuable information on the contribution of $I_{Ca(T)}$ and other channel currents, to the PKC-mediated effect, and to the overall response to agonists.

9. Endocrine cells. Enzymological and immunocytochemical analyses have shown rat anterior pituitary tissue to contain both β- and α-PKC subspecies (163). In both the GH_4C_1 rat pituitary cell line (4) and single rat anterior pituitary gonadotropes (211), PKC activation has been reported to enhance Ca^{2+} influx through L-type channels, although the effect on $[Ca^{2+}]_i$ is quite different in each case. In GH_4C_1 cells, which possess at least two different types of Ca^{2+} channels, PKC activators were found to produce an initial, transient elevation of $[Ca^{2+}]_i$ (that could be blocked by DHP antagonists), that was followed by a prolonged, slightly inhibitory effect. In contrast, thyrotropin-releasing hormone (TRH) produced a sustained enhancement of $[Ca^{2+}]_i$, the initial phase of which was also sensitive to DHP antagonists. It has been suggested, however, that the effects of TRH and PKC activators on I_{Ca} may be mediated indirectly through inhibition of I_K (58). In pituitary gonadotropes, gonadotropin-releasing hormone (GnRH) has been shown to cause two distinct phases of $[Ca^{2+}]_i$ elevation (211). The first of these is thought to be produced by 1, 4, 5-IP_3, as it is independent of extracellular Ca^{2+}, whereas the second phase results from Ca^{2+} influx through L-type channels, as it is inhibited by DHP antagonists. TPA was found to produce a small increase in $[Ca^{2+}]_i$, but to enhance markedly the secondary rise produced by GnRH.

PKC activators have been shown also to have inhibitory effects on Ca^{2+} currents in pituitary cells. By using the whole cell patch-clamp technique, OAG and phorbol esters were found to reduce rapidly and reversibly the Ca^{2+} current amplitude in GH_3 cells (149) and the adrenocorticotrophic hormone-secreting AtT-20 pituitary cell line (130; see also 135). In the former case, both the peak current ($I_{Ca(L)} + I_{Ca(T)}$) and the steady state current ($I_{Ca(L)}$ only) were reduced. By using a two-pulse voltage protocol to separate the $I_{Ca(L)}$ and $I_{Ca(T)}$ components of the whole cell Ca^{2+} current, it was found that both types of channel current were affected, to almost the same extent, implying that both are targets for PKC modulation. In these cells, $I_{Ca(L)}$ sustains the action potential, whereas $I_{Ca(T)}$ may play a part in its initiation. In AtT-20 cells, the effect of PKC was not altered by *Pertussis* toxin treatment, whereas a similar reduction of I_{Ca} by somatostatin was abolished completely by this treatment. This indicates that the effect of OAG on I_{Ca} is not mediated by modulation of G_i or G_o, and also implies a dissociation of the mechanism of action of somatostatin from a purely PKC-mediated one. In AtT-20 cells, PKA has a stimulatory effect on I_{Ca} (130). In general agreement with these results, in rat anterior pituitary tissue prisms preincubated with either TPA or OAG, luteinizing hormone-releasing hormone (LHRH)-stimulated $^{45}Ca^{2+}$ uptake was found to be reduced (157). An additional site of action of PKC in GH_3 cells that should be considered, however, has been suggested by the work of Drummond (57). PKC activators were found to accelerate the decay of the $[Ca^{2+}]_i$ rise promoted by TRH, but, in this case, it was concluded that the effect was not mediated by a modulation of I_{Ca}, but rather by stimulation of Ca^{2+} sequestration or its efflux.

Early studies by Knight & Baker (118) showed that TPA was capable of enhancing the Ca^{2+} sensitivity of catecholamine secretion from bovine adrenal medullary cells, although the site of action of PKC was unknown. In agreement with this, in perfused rat adrenal medulla, $^{45}Ca^{2+}$ uptake and secretion of catecholamines evoked by afferent stimulation, high-K^+ or nicotine was enhanced by pretreatment with phorbol esters, whereas that by muscarine was unaffected (240). These results suggest that Ca^{2+} influx through voltage-operated Ca^{2+} channels, and through nACh receptor-operated channels, can be enhanced by PKC in this tissue. In bovine adrenal chromaffin cells, however, TPA has been shown to be capable of inhibiting enkephalin synthesis, as a result of inactivating the L-type channels that are responsible for maintaining elevated $[Ca^{2+}]_i$ levels following high-K^+ depolar-

ization (193). Again, the discrepancies between the latter two reports are not readily explainable, but may be due to the methodologies employed, or the relative time course of the experiments.

10. Other tissues. Activators of PKC have been reported to increase $I_{Ca(L)}$ in mouse 3T3 and human fibroblasts (40) and the UMR-106 osteosarcoma clonal cell line (255). These observations are of particular interest because a number of types of fibroblast cell lines and the MC3T3-L1 osteoblast clonal cell line appear to express only the α-subspecies of PKC (see 'Introduction'; K. Hashimoto, unpublished observations), causally implicating this subspecies in the stimulatory modulation of $I_{Ca(L)}$. In UMR-106 cells, pretreatment with a low dose of TPA (parathyroid hormone or forskolin) blocked the subsequent stimulatory eflect of TPA, suggesting that an additional inhibitory modulation of the channel could also occur.

B. Potassium channels

The experimental findings describing the modulation of K^+ channel function by PKC are summarized in table 3.

1. Channel classification. Information on the primary structure of vertebrate K^+ channels is lacking at present, mainly due to their relatively low abundance and the associated difficulties in their purification. A *Drosophila* gene has been cloned, however, that encodes several distinct products, as a result of alternative splicing, capable of forming functional K^+ channels. The kinetic properties of these channels are similar to the species of channel carrying the rapidly inactivating 'A-current,' $I_{K(A)}$ (86). The encoded protein is structurally homologous to a single transmembrane domain of the Na^+ and L-type Ca^{2+} channels, and may form a functional ion channel by association of four such molecules (39).

Electrophysiological experiments have shown K^+ channel currents to be diverse in nature, varying widely in their kinetic properties, voltage dependence, pharmacology, sensitivity to modulation, and unitary conductance. They form the most ubiquitous of the ion channel groups, being found in fungi, animals, and plants, and in most cases, several different species are coexpressed within a single cell. Activation of K^+ channels is generally associated with an inhibitory response, as they mediate the outward movement of K^+ ions. As such, they are responsible for setting the resting potential across the plasma membrane, and, in excitable cells, its repolarization following an action potential, and the AHP, which modulates the frequency and threshold response of action potential propagation. Their classification has been reviewed thoroughly in recent articles (27, 44, 202), and so, the nomenclature used in this review is based on the information contained therein. A brief definition of the terminology used is given at first mention below, but readers are advised to consult the latter papers for a more detailed explanation of the properties of the various types of channel. Where classification of a channel within this framework is unclear, the nomenclature used in the original article is adopted.

2. Invertebrate photoreceptors. Exogenous application of PDBu, or intracellular injection of a partially purified, unresolved mixture of PKC subspecies from rat brain into *Hermissenda* Type B photoreceptors has been shown to result in the persistent reduction of two types of K^+ current, $I_{K(A)}$ and $I_{K(Ca)}$ [a type of Ca^{2+}-dependent K^+ current; there appear to be at least three distinct species of this type of current: $I_{K(Ca(B))}$, mediated by a high ('big') conductance channel, also called I_C; $I_{K(Ca(I))}$, mediated by an 'intermediate' conductance channel; and $I_{K(Ca(S))}$, mediated by a low ('small') conductance channel, also called I_{AHP}] (63). These changes, together with an enhancement of I_{Ca} (section II A, table 2), result in the prolonged elevation of $[Ca^{2+}]_i$, in a manner similar to that observed following the temporally associated stimuli, light, and rotation. Injection of PKA into these cells failed to reduce either $I_{K(A)}$ or $I_{K(Ca)}$, but reduced another, unidentified K^+ current (63). Pharmacological activation of endogenous PKC in *Hermissenda* Type B photoreceptors was also reported by Alkon et al. (5) to reduce persistently these two K^+ currents, but only when accompanied by an elevation of $[Ca^{2+}]_i$. A more recent paper by the latter group has shown that, in contrast to the results of Farley & Auerbach (63), iontophoretic injection of PKC alone resulted in an increase in $I_{K(A)}$ and $I_{K(Ca)}$ whereas injection of the active enzyme into cells preexposed to DPBA, or following a 'Ca^{2+}-load' paired with a conditioned stimulus (light), caused prolonged reduction of these K^+ currents, without affecting currents carried by Ba^{2+} (6). These effects were found to be mimicked by iontophoretic injection of the protease inhibitor leupeptin. In addition, prior injection of leupeptin could transform the stimulatory effect of PKC injection on $I_{K(A)}$ and $I_{K(Ca)}$ to an inhibitory one. As a result of these studies, it has been proposed that the persistent inhibitory effect of PKC is the result of the prolonged association of an activated form of the enzyme with the membrane [possibly by a mechanism similar to that advanced by Bazzi & Nelsestuen (19), although this is not at all clear at present], whereas the stimulatory response is mediated by a cytosolic form of the enzyme, possibly modified by protease action.

3. Invertebrate neurones. Pretreatment of *Aplysia* bag cell neurones (internally dialyzed with buffer containing EGTA to block any Ca^{2+}-dependent K^+ currents) with TPA, or intracellular injection of a purified, but unresolved, mixture of PKC subspecies from bovine brain, failed to have any effect on $I_{K(A)}$ or any of the other voltage-operated K^+ currents present in this cell (52). In the same cell, activation of PKA was found to have a converse effect to PKC, by decreasing at least three K^+ currents, without affecting the inward current (109, 225,

218). Application of the neuropeptide CCK-8 to the identified neurones F77 and D2 from *Helix aspera* was found to be ineffective at reducing the TEA-insensitive, Ca^{2+}-independent outward current in these cells, whereas the same treatment resulted in the inhibition of a voltage-operated Ca^{2+} conductance (80; section II A, table 2).

4. Vertebrate central nervous system neurones. Intracellular recording from CA1 pyramidal neurones in a slice preparation of rat hippocampal tissue, during bath application of 4β-phorbol 12,13-diacetate (PDA) or PDBu, has shown that these agents are able to promote a long-lasting inhibition of the Ca^{2+}-dependent K^+ current, $I_{K(Ca(S))}$, that underlies the slow AHP of these neurones following current-induced activation (14, 144, 224). As a consequence, the spike frequency adaptation or 'accomodation' which normally occurs with depolarizing stimuli was reduced, allowing an enhanced rate of action potential discharge. This type of response had previously been reported to be evoked in CA1 cells by an increase in cAMP, as a result of activation of β_1-adrenergic and H_2-histamine receptors (138, 78), indicating that the channel carrying the $I_{K(Ca(S))}$ current can be modulated by either system to produce essentially the same effect. Inhibition of the slow AHP by agonists using the cAMP pathway appears to be incomplete, however, and more readily reversible than that induced by phorbol ester activation of PKC. Under the same conditions, the fast AHP, which is mediated by another species of Ca^{2+}-dependent K^+ current, $I_{K(Ca(B))}$, and is important for the repolarization of the action potential, is not affected. This suggests that PKC is specifically targetting $I_{K(Ca(S))}$, and is not doing so indirectly by inhibiting Ca^{2+} influx. This conclusion is supported by the observation that the Ca^{2+}-dependent action potential was not reduced by phorbol ester treatment, and in some experiments was slightly increased (14, 144; section II A, table 2). The latter two papers reported no change in the amplitude or duration of each action potential following phorbol ester treatment, whereas Storm (224), found a broadening of the action potential as a result of inhibition of the repolarizing phase.

Acetylcholine, acting at M_1-muscarinic receptors (60; but see 43), is capable of reducing $I_{K(Ca(S))}$ in CA1 cells, and in addition, is able to block $I_{K(M)}$ ('M-current'). Although PKC activation is known to inhibit $I_{K(M)}$ in NG108-15 and sympathetic neurones (see below), it has been reported not to have an effect on this channel in CA1 cells (144). This raises two interesting points: firstly, that $I_{K(M)}$ is sensitive to PKC activation in some cell types, but not others, and, secondly, that it is susceptible to modulation by different intracellular messengers. In fact, it has been suggested that in CA1 cells, the cholinergic inhibition of $I_{K(M)}$ is due to the activation of another species of muscarinic receptor, and may be mediated by an effect of 1, 4, 5-IP_3, other than through the release of intracellular Ca^{2+} (59, 60). This mechanism, however, has been discounted in rat and frog sympathetic neurones (32, 191).

As mentioned in an earlier section, hippocampal CA1 cells also express a species of Ca^{2+}-independent K^+ current that is selectively increased by the $GABA_B$ receptor agonist baclofen, and by serotonin, acting at 5-HT_{1A} receptors (7). In this case, but not in the M_1-muscarinic receptor blockade of $I_{K(Ca(S))}$ (60), the ion channel is directly coupled to the receptor by a *Pertussis* toxin-sensitive G-protein. PDBu was found to reduce greatly the response to both serotonin and baclofen. Therefore, in this cell, two distinct species of K^+ channels, that differ in their ability to interact with G-proteins, are both targets of PKC.

In another study, using acutely dissociated guinea-pig hippocampal neurones and rat fetal hippocampal neurones, various PKC activators were found to suppress two types of persistent K^+ currents, $I_{K(Ca)}$, and the delayed rectifier current, $I_{K(A)}$ (a voltage-operated current activating at relatively positive potentials), without affecting the transient current, $I_{K(A)}$ (54). The effects of the PKC activators were reversible with washing, and could be inhibited by H-7 or polymixin B, independent of any action on PKA or the Ca^{2+}/calmodulin-dependent protein kinase. Under the same experimental conditions, PKC activators were also found selectively to affect components of I_{Ca} (section II A, table 2).

There is now increasing evidence to suggest the involvement of PKC subspecies at some stage(s) of the processes underlying the enhancement of synaptic efficiency at the synaptic contacts between Schaffer collateral/comissural fibers and the dendritic spines of hippocampal CA1 pyramidal cells, termed long-term potentiation (LTP) (see 167, 110, 148). From a biochemical point of view, hippocampal LTP has been reported to coincide with a translocation of PKC from the cytosol to the membrane (3), resulting in the increased phosphorylation of several presynaptic membrane proteins, such as F1 (synonymous with B-50 and GAP-43; 134, 174, 23), whereas classical conditioning in the rabbit has been reported also to result in the persistent translocation of PKC, possibly to the postsynaptic membrane of the proximal dendrites of CA1 cells (13). Intracellular electrophysiological recording from rat hippocampal slices superfused with β-phorbol esters (145), or following injection of a purified mixture of PKC subspecies into CA1 cells (93), revealed these experimental manipulations to evoke a response qualitatively similar to that of LTP, i.e., an increase in the amplitude of the excitatory postsynaptic potential (e.p.s.p.; an index of the dendritic synaptic response, measured as a net inward under voltage-clamp conditions), an increase in the probability of action potential discharge, an increase in the population spike (an index of the synchronous activation of pyramidal cells), and a decrease in the spike latency. Although

TABLE 3

Modulation of potassium channel activity by protein kinase C activation

Cell type	Recording method*	Stimulus	Current response	Reference
Hermissenda B photoreceptors	*a*	PDBu (1–10 nM) PKC injection	$\downarrow I_{K(A)}$ $\downarrow I_{K(Ca)}$	63
	a	OAG (2.4–120 μM) DPBA (1–100 nM) PKC injection	$\downarrow I_{K(A)}$ $\downarrow I_{K(Ca)}$	6
Aplysia bag cell neurones	*a*	TPA (1–100 nM) PKC injection	$\rightarrow I_K$ $\rightarrow I_{K(A)}$	52
Helix aspera neurones	*a*	CCK-8 (5 nM)	$\rightarrow I_K$	80
Rat hippocampal CA1 pyramidal neurones	*a*	PDA (250 nM) PDBu (100 nM)	$\downarrow I_{K(Ca(S))}$	14
	a	PDBu (10 μM)	$\downarrow I_{K(Ca(S))}$ $\rightarrow I_{K(M)}$	144, 59, 60
	a	PDBu (5–10 μM) PDA (300 nM-5 μM)	$\downarrow I_{K(Ca(S))}$	224
Guinea pig and rat fetal hippocampal pyramidal neurones	*a*	PDA (1.5 μM) PDBu (2 μM) OAG (60 μM)	$\downarrow I_{K(V)}$ $\downarrow I_{K(Ca)}$ $\rightarrow I_{K(A)}$	54
Rat dorsal raphe neurones	*a*	PDBu (10–100 nM) PDA (100–500 nM)	$\uparrow I_K$ $\downarrow I_{K(Ca)}$	71, 72
Rat whole brain extract (reconstituted)	*f*	PKC application	$\downarrow I_{K(Ca(I))}$ $\uparrow I_{K(type\ III)}$ $\rightarrow I_{K(Ca(B))}$	132
Rat hippocampal and striatal synaptosomes	*d*	DiC_8 (15–150 μM) OAG (150 μM)	$\downarrow I_{K(A)}$ $\downarrow I_{K(V)}$ $\rightarrow I_{K(Ca)}$	42
Cultured primary astrocytes	*g*	TPA (100 nM) DiC_8 (50 μM) Phenylephrine (80 μM)	$\downarrow I_K$	2
Cultured ovine oligodendrocytes	*a*	TPA (1.5–600 nM)	$\downarrow I_{K(A?)}$ $\downarrow I_{K(V?)}$	221
Mouse dorsal root ganglion and cerebral hemisphere neurones	*a*	TPA (1–100 nM) PDBu (10 nM–1 μM)	$\downarrow I_{K(A)}$ $\downarrow I_{K(V)}$	243, 244, 76
Chick dorsal root ganglion neurones	*a*	OAG (600 nM–60 μM) DPBA (10 nM–50 μM)	$\rightarrow I_K$ $\rightarrow I_{K(Ca)}$	194
Frog sympathetic ganglion neurones	*a*	PDBu (100 nM–30 μM)	$\downarrow I_{K(M)}$	1, 33
	a	OAG (50–75 μM) PDBu (1 μM)	$\downarrow I_{K(M)}$	234
Frog sympathetic neurones	*a*	TPA (1 μM)	$\downarrow I_{K(M)}$	191
NG108-15 neuroblastoma × glioma cell line	*a*	PDBu (10 nM–1 μM) Bradykinin (10 μM)	$\downarrow I_{K(M)}$ $\rightarrow I_{K(Ca(S))}$ $\rightarrow I_{K(V)}$	85, 34, 35
Guinea-pig cardiac ventricular myocytes	*a*	TPA (1–100 nM) OAG (125 μM)	$\uparrow I_{K(V)}$	231
	a	PDBu (10 nM) TPA (100 nM)	$\uparrow I_{K(V)}$	242
Rat cardiac ventricular myocytes	*a*	TPA (10 nM–1 μM) OAG (60 μM) Phenylephrine (5 μM) Methoxamine (5–10 μM)	$\downarrow I_{K(A)}$ $\downarrow I_{K(V)}$	8
RINm5F insulin-secreting cell line	*b*	TPA (100 nM) DiC_{10} (12.5 μM)	$\downarrow I_{K(ATP)}$	246
AtT-20 mouse pituitary cell line	*a*	OAG (50 μM)	$\rightarrow I_K$	130
Xenopus oocyte	*a*	TPA (5–20 nM) PDBu (20–100 nM) Acetylcholine (1 μM)	$\downarrow I_K$	51

* Recording methods: *a*, whole cell recording under current- or voltage-clamp configuration; *b*, cell-attached membrane patch voltage-clamp; *d*, ion flux measurements; *f*, artificial lipid bilayer formed on patch pipette tip; *g*, measurement of membrane potential by absorbance of a cyanine dye. Abbreviations and channel nomenclature as given in the text.

both approaches provide pharmacological evidence for its involvement, the site of action of PKC is still a matter of discussion. Malenka and colleagues (145) concluded that the effect of phorbol esters was mediated at a presynaptic locus (possibly by facilitating glutamate release; see also 146), whereas Hu and colleagues (93) concluded that the injected PKC was working at a dendritic locus, since the changes induced by PKC injection were observed only when the cells were activated synaptically. Hippocampal tissue is known to contain at least four subspecies of PKC (α, βI, βII, and γ; 212, 96, 99), but expresses high levels of γ-PKC and almost no βI-PKC. Immunohistochemical analysis with monoclonal antibodies has shown that the pyramidal cell bodies and apical dendrites stain heavily with anti-γ PKC, whereas this subspecies is apparently not present in the presynaptic terminal (204; see also 159, antibody 'CK1.12'). Using subspecies-selective antibodies, CA1 pyramidal cells have also been reported to contain β- and α-PKC (97, 99, 263). On the other hand, polyclonal antisera raised against a mixture of subspecies identified dense immunoreactive material associated with synaptic vesicles in the presynaptic terminals adjacent to the pyramidal cell apical dendrites in the CA3 region (247). In this case, if the formation of LTP actually does involve both pre- and postsynaptic components, then different PKC subspecies are likely to be involved. Whether or not modulation of ion channel activity, such as the $I_{K(Ca(S))}$ and $I_{K(V)}$ currents (described above) or voltage-operated Ca^{2+} channels (table 2), plays a part in this process, remains to be determined.

Similar net increases in the neuronal excitability, without significant changes in the resting membrane potential or input resistance, have been reported in cat spinal motoneurones (259) and cat motor cortex neurones (16), following injections of PDA or TPA/PDBu into the cell soma of anaesthetized or awake cats, respectively. In both cases, intracellular recording in situ monitored a gradually developing, but persistent, increase in the–predominantly Na^+-dependent–action potential amplitude, without changes in its duration, and an increase in the amplitude of the fast AHP. Even with the latter effect (not observed in CA1 cells), accomodation was reduced, and action potential bursting activity developed with time. In cat motor neurones, the slow AHP was completely abolished, as described for hippocampal cells. At the synapse level, stimulation of afferent fibers following injection of PDBu into motor cortex neurones was found to result in an increase in the amplitude and duration of the e.p.s.p., and also an increase in the inhibitory postsynaptic potential (i.p.s.p., a net outward current) (17). By analogy with other systems, the former effect was speculated to be due to the inhibition of $I_{K(A)}$, and the latter effect could involve inhibition of Ca^{2+}-dependent K^+ channel currents and an inward chloride (Cl^-) current. The validity of these speculations is likely to be reported in the near future.

Intracellular recording from serotonergic neurones of the rat dorsal raphe revealed that the net effect of the phorbol esters PDBu and PDA was a hyperpolarization of the cell (71, 72). This was proposed to result from PKC activation of an outward current, probably carried by K^+ ions, coupled with inhibition of I_{Ca} (section II A, table 2). The latter effect probably results in the inhibition of $I_{K(Ca)}$, accounting for the observed reduction of the spike AHP. In contrast, α_1-adrenergic receptor agonists are known to promote cell depolarization, which appears to be subject to feedback inhibition by PKC. This study provides a good illustration of how the net effect on the cell membrane potential can be different following selective stimulation of the PKC pathway by phorbol esters, compared with stimulation of both arms of the Ca^{2+}/PKC signal pathway using an agonist.

Rudy and colleagues (64, 132, 196) have used the novel and rather promising technique of reconstituting K^+ channels from rat brain synaptosomes into artificial lipid bilayers formed on the tips of microelectrodes, to study the effects of protein kinases on their conductance properties. Using this technique, bath application of PKC resulted in a decrease in the open-time of a Ca^{2+}-dependent K^+ channel, of intermediate size current amplitude, resembling $I_{K(Ca(I))}$ (called 'type II'), but an increase in the open-time of another voltage- and Ca^{2+}-independent K^+ channel, with a smaller current amplitude, $I_{K(type\ III)}$. PKC application was found to be without effect on $I_{K(Ca(B))}$ (called 'type I'). In contrast to the effects of PKC, bath application of the catalytic subunit of PKA had essentially the opposite effect, stimulating an increase in $I_{K(Ca(I))}$ and $I_{K(Ca(B))}$, but with no consistent effect on $I_{K(type\ III)}$.

Using tracer efflux studies in rat hippocampal and striatal synaptosomes as a model for studying the modulation of presynaptic K^+ channel activity by PKC, Colby & Blaustein (42) found that DiC_8 reduced, in a time- and concentration-dependent manner, $^{86}Rb^+$ efflux through voltage-operated K^+ channels, with the kinetic and pharmacological characteristics of $I_{K(A)}$ and $I_{K(V)}$, but was ineffective on $I_{K(Ca)}$. The inhibitory response of DiC_8 could be attenuated by H-7 and sphingosine, providing supportive evidence for a role of PKC. On the basis of these findings, it was suggested that an increase in neurotransmitter release stimulated by DAG analogues was the result of a block of these K^+ channels, which would prolong the depolarization of the nerve terminal and increase the entry of Ca^{2+} through voltage-operated Ca^{2+} channels. This is a plausible mechanism. Under the same experimental conditions, however, OAG was found to be less effective than DiC_8, and TPA, PDBu, PDA, and DPBA were reported to be without activity. This observation is difficult to reconcile with the findings for DiC_8, as it is known that phorbol esters are potent activators

of PKC in vitro, can stimulate the translocation of PKC from the cytosol to the membrane in synaptosomes, have been shown to be effective at modulating these particular K^+ channel currents in other cells (see table 3), and are known to stimulate neurotransmitter release from synaptosomes. Further clarification of the relative activity of these compounds at inhibiting K^+ channel function in this system should, therefore, be attempted.

5. Vertebrate central nervous system glial cells. In addition to the extensive studies carried out on the effects of PKC activation on neuronal ion conductance, recent work has been directed toward the role of PKC in glial cells, where an enzymatic activity of PKC (158, 160) and immunohistochemical detection has been achieved (see, for example, 170). MacVicar et al. (137) have shown that TPA induces endogenously generated rhythmic oscillations of the membrane potential of cultured astrocytes from kainic acid-lesioned slices of the hippocampal CA3-CA4 region. The oscillatory activity was not dependent on the influx of Ca^{2+} through voltage-operated Ca^{2+} channels, but was associated with an increased input resistance during the depolarizing phase, that could be accounted for by an inhibition of I_K. TPA-induced depolarization of the membrane potential of cultured astrocytes (mimicked by DiC_8 and phenylephrine) has been observed also by Åkerman et al. (2), who, on the basis of a comparison with the actions of valinomycin and gramicidin, conclude that PKC activation results in an inhibition of I_K. In cultured oligodendrocytes, whole cell patch clamp analysis also has shown TPA to decrease I_K, which could be separated into a transient component (resembling $I_{K(A)}$), and a steady state component (resembling $I_{K(V)}$) (221). The amplitude of both outward currents was reduced by TPA [but not by the inactive phorbol ester, 4α-phorbol 12,13-didecanoate (4α-PDD)], although the steady state current appeared to be more susceptible. The effect of TPA was mimicked by forskolin (25 μM), suggesting that the channels involved are common substrates for PKC and PKA. In this cell type, however, the effect of TPA and forskolin was found essentially to be irreversible, which, with the present knowledge, complicates the formation of a general model in which PKC modulation of I_K contributes to the rapid oscillation of the membrane potential observed in astroglial cells. Nevertheless, the receptor-linked modulation of glial cell ion channel function via PKC (and/or PKA) is an important aspect of future research, as these cells play a crucial role in controlling the homeostasis of the extraneuronal ionic environment, which in turn, may have a profound effect on neuronal excitability.

6. Vertebrate peripheral neurones. Application of PDBu or TPA produced changes in the action potential duration of mouse DRG cells that were dependent on the holding potential (243, 244). When the cells were held at the resting potential or hyperpolarized (−65 to −100 mV), phorbol esters prolonged the action potential duration, and partially decreased the spike AHP, whereas at less negative potentials, the action potential duration was decreased. Using the single electrode voltage-clamp mode, in the presence of cadmium ions to block Ca^{2+} channels, it was found that phorbol esters reduced both an early current, blockable by aminopyridines, probably $I_{K(A)}$, and a late current, blockable by TEA, probably $I_{K(V)}$. The latter channel current was also reduced in these cells by the application of forskolin, suggesting that it, or some regulatory protein associated with it, is a common substrate for both PKC and PKA (76). The effect of phorbol esters on $I_{K(A)}$ and $I_{K(V)}$ in mouse DRG and cerebral hemisphere pyramidal neurones was readily reversible (3–5 min). In contrast to these results, application of either OAG or DPBA to embryonic chicken DRG neurones grown in culture was found not to change the current through either voltage-operated or Ca^{2+}-dependent K^+ channels, under conditions which caused a substantial reduction of I_{Ca} (194; section II A, table 2).

In frog sympathetic neurones (1, 33, 234, 191) and the hybrid NG108–15 cell line (85, 34, 35), PKC activation by phorbol esters or agonists acting through receptors coupled to PIP_2 hydrolysis (such as the M_1-muscarinic and bradykinin receptors) results in a persistent reduction of $I_{K(M)}$, leading to cell depolarization. PKC-mediated reduction is submaximal, however, leaving a residual component that is susceptible to further reduction by application of agonist. The finding that the direct activation of PKC is only partly able to replicate the effects of agonists has been interpreted as indicating the involvement of an additional regulatory molecule, although, as discussed above, in these cell types the candidacy of 1, 4, 5-IP_3 as the additional messenger has been dismissed. Tsuji et al. (234) have reported that, in frog sympathetic ganglion neurones, the PKC-mediated reduction of $I_{K(M)}$ is accompanied by the opening of a nonselective cation channel ('D-channel'), both of which contribute to the generation of the slow E.P.S.P. The efficacy of the muscarinic-induced opening of the D-channel was found to be approximately 10-fold lower than that for the inhibition of $I_{K(M)}$ which has prompted these authors to suggest that this is a hierarchical mechanism for determining the order in which the channels are regulated. If receptor occupancy is directly related to PKC activation, then this represents an additional mechanism whereby an extracellular signal can encode the differential modulation of ion channel activity. Other mechanisms already encountered are the temporal modulation of $I_{K(Ca(S))}$ and $I_{K(M)}$ by 1, 4, 5-IP_3 and PKC (85), and the interaction of receptors and G-proteins to provide convergent and divergent signalling systems, as recently discussed by Nicoll (166).

In NG108–15 cells, PDBu was found to be without effect on $I_{K(Ca(S))}$, but could augment the stimulatory action of 1, 4, 5-IP_3 on this current (35). Bradykinin,

under conditions that completely abolished $I_{K(M)}$, was found to be without effect on $I_{K(V)}$ (34).

7. Cardiac muscle cells. Superfusion of guinea pig ventricular myocytes with TPA or OAG (231) and PDBu or TPA (242) has been reported to increase the amplitude of $I_{K(V)}$. The response to TPA application is further enhanced during the intracellular dialysis of a purified preparation of type III(α) PKC from bovine brain (N.Tohse et al., unpublished observations), the major PKC subspecies isolated from heart tissue (120). The effect of TPA was found to be temperature dependent, being observed at 32°C, but not at 22°C (242), to require the presence of nanomolar concentrations of free Ca^{2+}, and be inhibited by H-7 (231). Ca^{2+} ions are also able to enhance $I_{K(V)}$, apparently independently of kinase enzyme activation, as this enhancement is not blocked by H-7 (231). Phorbol esters and DAG analogues have been shown previously to have rapid, dose-dependent negative effects on the inotropic and chronotropic response of isolated, perfused rat heart (258). The above electrophysiological findings for PKC-mediated modulation of $I_{K(V)}$ could underlie these changes. An increase in $I_{K(V)}$ would lead to a shortening of the action potential duration, that would in turn decrease the Ca^{2+} influx during the plateau phase, and, hence, result in a decrease in contractile force.

In contrast to these two reports, in rat ventricular myocytes, application of TPA, OAG, or the adrenoceptor agonists phenylephrine and methoxamine (acting at the α_1-receptor subtype) have been reported to produce an increase in the action potential duration by partial inhibition of $I_{K(A)}$ and $I_{K(V)}$ currents (8). The effect of the PKC activators was found to be rapid and reversible, specific to I_K, and of the two currents, $I_{K(A)}$ was found to be the most susceptible (compare the results of Doerner et al. (54) in the hippocampus). Clearly, the observations in rat ventricular myocytes do not agree with those described above for guinea pig ventricular myocytes. Some genuine species differences may occur, but the relative contribution and importance of PKC-mediated changes in I_K and I_{Ca} to the inotropic effects in cardiac cells still must be considered as very contentious. A careful dissection of the individual components of any current recording is vital in this respect.

8. RINm5F insulin-secreting B-cell line. Recent biochemical analysis has shown rat whole pancreas tissue predominantly to express the α-subspecies of PKC, with the β-subspecies being a minor component (approximate activity ratio, 11%:89%). The minor enzyme fraction resolved by hydroxyapatite column chromatography almost exclusively contains the βII-subspecies, and immunohistochemical staining of serial sections of the pancreas has revealed this enzyme immunoreactivity to localize only in the B-cells (265). This observation suggests that the βII-subspecies of PKC is involved in a function specific to these cells, possibly related to the secretion of insulin. The generally accepted mechanism by which carbohydrates depolarize pancreatic B cells and stimulate insulin secretion is via a decrease in K^+ current with a consequent opening of voltage-operated Ca^{2+} channels, and an increase in $[Ca^{2+}]_i$. The ability of TPA to enhance the secretory response of islets to glucose or sulphonylureas, has been known for some time (143), but the site of action of PKC remained to be clearly established. In a recent report, using the RINm5F cell line, single channel recording in the cell-attached membrane patch configuration has shown that TPA and the synthetic DAG, 1,2-didecanoylglycerol (DiC_{10}), cause a rapid decrease in the open probability and single channel current amplitude of two types of ATP-sensitive K^+ channels, in a fully reversible manner (246). This results in a transient depolarization of the cell, an increase in $[Ca^{2+}]_i$ due to influx through voltage-operated L-type Ca^{2+} channels, and consequently, insulin secretion, mimicking the effect of the endogenous activator, glyceraldehyde. In these cells, however, the rise in $[Ca^{2+}]_i$ effected by PKC mediated cell depolarization alone, has been found to be significantly less than that by glyceraldehyde or KCl (246, 254), but the release of insulin greater (254). These observations may be due, respectively, to a secondary inhibitory action of PKC on $I_{Ca(L)}$, as reported by Meldolesi and colleagues (53; section II A, table 2), and an additional effect on some part of the secretory mechanism, as is apparent in PC 12 cells. The results described above probably also explain the enhanced Ca^{2+} current, through L-type channels, observed in these cells by Velasco (238), following exposure to glyceraldehyde or DiC_{10}.

9. Other tissues. In AtT-20 cells, PKC activation has been reported not to have an effect on I_K, under conditions where a voltage-operated Ca^{2+} current ($I_{Ca(L)}$?) was reduced (130; section II A, table 2).

In the *Xenopus* oocyte, adenosine induces a K^+ conductance increase, I_K, that is fully mimicked by intracellular application of cAMP. Acetylcholine, acting at a muscarinic cholinergic receptor, was found to decrease the K^+ conductance increase evoked by adenosine, and also by a phosphodiesterase inhibitor and intracellular cAMP, suggesting that the effect was not through inhibition of adenylate cyclase (51). The inhibitory action of acetylcholine was found not to be mimicked by Ca^{2+} or 1, 4, 5-IP_3, but was by TPA or PDBu, suggesting an involvement of PKC. In contrast to the effect of acetylcholine, which was reversible, the inhibitory effect of TPA was found to be essentially irreversible.

C. Other Channels

Although by far the majority of studies investigating the effects of PKC activation on ion channel function have focussed on the measurement of I_{Ca} or I_K, there are a number of reports of the conductance properties of

other types of channels being affected. The experimental findings are summarized in table 4.

1. Vertebrate central nervous system neurones. In rat hippocampal pyramidal cells, but as yet, no other CNS neurones, prolonged phorbol ester treatment has been reported to inhibit a time-dependent and voltage-operated inward Cl^- current, I_{Cl}, that is activated by hyperpolarization, but is insensitive to nACh receptor stimulation (139). Although the recording conditions employed did not allow a completely reliable prediction of its location, it was suggested that the channel carrying this current was present in the dendritic membrane. Inhibition of the current by PKC could be responsible, therefore, for enhancing the transmission of excitatory events from the dendrite to the cell body. The rate of action potential discharge in these cells is thought to be modulated also by the inhibitory action of PKC on $I_{K(Ca(S))}$ (section II B, table 3).

Injection of total mRNA isolated from chick forebrain into *Xenopus* oocytes led to the expression of functional voltage-operated Na^+ (I_{Na} current) and Ca^{2+} channels, of ligand-operated $GABA_A$ ($I_{Cl(GABA)}$ current) and kainate ($I_{M^+(kainate)}$ current) receptor channels, and of quisqualate receptors that could activate endogenous Cl^- channels by means of an 1, 4, 5-IP_3-mediated rise in $[Ca^{2+}]_i$ (219). Exposure of the oocytes to either TPA or OAG (but not inactive phorbol esters) was found to cause a progressive decrease in the amplitude of I_{Na} and $I_{Cl(GABA)}$ (and also I_{Ca}; section II A, table 2), whereas $I_{M^+(kainate)}$ was unaffected. The effects of the PKC activators were mimicked by quisqualate, and could be inhibited by tamoxifen. With the reservations expressed above about the interpretation of the results of this type of experiment kept in mind, these findings provide further evidence of the susceptibilty of multiple classes of ion channels coexpressed within the same cell, to modulation by Ca^{2+} and PKC. The electrophysiological data on I_{Na} and $I_{Cl(GABA)}$ are novel findings for central nervous system-derived channels. Differential phosphorylation of the α-subunit of the purified Na^+ channel from rat brain, and the native Na^+ channel in synaptosomal membranes by PKC (45) and PKA (46) was demonstrated some time ago, but the outcome of this reaction on channel activity remained unknown.

2. Vertebrate peripheral neurones. Whole cell voltage-clamp analysis of rat DRG sensory neurones exposed to PDBu showed that a proportion of the neurones tested (approximately 50%) responded with an inward current that was tentatively identified as an enhanced Cl^- conductance (131). A similar increase in a proportion of the neurones was also found to be elicited by bradykinin.

Changes in the resting potential and action potentials of nonmyelinated sensory fibers (C-fibers) of the rat vagus nerve have been recorded in response to phorbol ester treatment. Activation of PKC produced a strong depolarization that appeared to result mainly from an increase in I_{Na}, although an outward Cl^- conductance may also be involved (195). The regulation of I_{Na} was found to be unusual in that it was affected by divalent cations and enhanced at lower pH.

3. Muscle cells. In mammalian muscle, Cl^- channels are responsible for most of the ionic conductance of the membrane at the resting potential, and thus, the electrical stability of the membrane. Myotonia in muscle results from defects in this conductance. Exposure of mouse skeletal muscle fibers to PDA, or 4β-phorbol 12,13-didecanoate (4β-PDD) was found to result in the initiation of a 'run' of action potentials following a hyperpolarizing stimulus pulse, that was associated with a persistent decrease in I_{Cl}, without changing the resting potential, the amplitude of the action potentials, or the K^+ current (31). Bryostatin 1, a known activator of PKC, was even more active in this respect.

In the A_7r_5 vascular smooth muscle cell line, cell-attached patch-clamp recording has revealed a large conductance, voltage-dependent, anion-selective channel (most likely I_{Cl}), that is activated only in the excised patch mode, implying that under normal conditions a tonic inhibitory mechanism acts on this channel, as found for I_{Cl} in other systems (220). Application of micromolar concentrations of H-7 to these cells resulted in the disinhibition of the Cl^- channels in a proportion of the patches tested (203). These results suggest that one of the mechanisms that tonically inhibits the resting I_{Cl} in vascular smooth muscle cells is phosphorylation by a kinase enzyme, possibly PKC.

III. Summary and Conclusions

The considerable volume of data now available strongly implicates the modulation of ion channel activity as a key function of the PKC enzyme family. In some systems, such as the PC 12, RINm5F, and NG108–15 clonal cell lines, the action of the enzyme is becoming clear, whereas in others, such as cardiac and smooth muscle cells, contradictory evidence exists, and clarification of the role of PKC in these tissues must await further analysis. Few general statements can be made concerning the regulation of ion conductance through the various classes of channel, or through one particular species of channel that is expressed in and subject to regulation in different cell types. Rather, it seems that many different patterns of modulation can occur in different cells, and so, elucidation of the molecular mechanisms that determine these diverse patterns represents a major challenge. To summarize, we will focus on three aspects of the modulatory action of PKC.

A. Nature of Channel Current Modification

In the limited number of reports where it has been determined, changes in channel current amplitude are usually the result of an alteration of the open probability

TABLE 4

Modulation of nonpotassium, noncalcium channel activity by protein kinase C activation

Cell type	Recording method*	Stimulus	Current response	Reference
Rat hippocampal pyramidal cells	*a*	PDBu (10 μM) PDA (10 μM)	↓ I_{Cl}	139
Xenopus oocyte expression system (chick brain mRNA)	*a*	TPA (10 nM) OAG Quisqualate (10 μM)	↓ I_{Na} ↓ $I_{Cl(GABA)}$ → $I_{M+(Kainate)}$	219
Rat dorsal root ganglion neurones	*a*	PDBu (1 μM)	↑ I_{Cl}	131
Rat vagus nerve (C-fibres)	*e*	PDBu (10 nM–1 μM)	↑ I_{Na}(↑ I_{Cl})	195
Mouse skeletal muscle fibres	*a*	PDA (1.6, 16 μM) PDD (5 μM) Bryostatin 1 (200 nM)	↓ I_{Cl}	31
A_7r_5 vascular smooth cell line	*b*	-	↓ I_{Cl}	203

* Recording methods: *a*, whole cell recording under current- or voltage-clamp configuration; *b*, cell-attached membrane patch voltage-clamp; *e*, grease-gap recording. Abbreviations and channel nomenclature as given in the text.

of the channel, rather than the single-channel conductance, current-voltage relationship of activation or inactivation, or reversal potential. In two reports (*Aplysia*, 52; skeletal muscle, 165), however, the number of functional channels in the membrane apparently was increased following PKC activation, and in one report (DRG cells, 208), the proportion of cells expressing a particular species of ionic current was decreased following prolonged exposure to TPA. The possible recruitment or down-regulation of channels has not been investigated in a range of cells, but it represents a very interesting phenomenon in terms of a mechanism for enhancing the capacity of a cell to respond to certain stimuli.

The selective up- or down-modulation of Ca^{2+} channel currents is now well established (233). Ca^{2+} channel current modulation by PKC seems to show a strong tissue dependency, with mostly stimulatory (smooth muscle) or mostly inhibitory (sensory neurones, PC 12 cells) responses being reported, whereas in the majority of tissues examined, K^+ and Cl^- channel activity is decreased (the $I_{K(V)}$ current of guinea pig cardiac ventricle cells is an exception to this). In certain tissues, one class of ion channel is susceptible to modulation whereas another is not (for example, Ca^{2+} channel versus K^+ channel in *Aplysia* and *Helix aspera* neurones) and selective targetting of one species of ion channel current can occur within a cell (for example, $I_{K(Ca(S))}$ in hippocampal CA1 cells) or in one cell type and not another (for example, $I_{K(M)}$ in NG108–15 cells and hippocampal CA1 cells).

As yet, a mechanism to explain the molecular basis for the differential effects of PKC activation has not been established. Single channel recording studies on isolated membrane patches have indicated that the changes in conductance properties are likely to result from either direct phosphorylation of the principal channel protein, or a closely associated polypeptide. The principal channel polypeptides of skeletal muscle voltage-operated L-type Ca^{2+} channels and brain voltage-operated Na^+ channels have been shown to be substrates for the enzyme in vitro, but the extent to which these, or other auxillary polypeptides associated with them, are phosphorylated following PKC activation in the intact cell is still unknown (a reliable demonstration of such an action of the enzyme will be extremely difficult). Factors that could determine the effect on conductance properties are the location of the acceptor amino acid(s) within the channel structure or the stoichiometry of phosphorylation of the different channel components. Selective targetting of channels may be the result of the action of different subspecies of PKC or the heterogeneity of the amino acid sequences of the ion channel components. An example of the latter mechanism has been provided by studies on the PKA-mediated modification of Na^+ channel function in different cells. The major phosphorylation sites on the brain Na^+ channel were located to a segment of the principal channel polypeptide that is absent from the equivalent protein of the electroplax Na^+ channel (39). Examination of the primary sequences of channels expressed in different tissues, and determining the phosphorylation sites on these proteins are important areas of future research.

B. Duration of PKC Effect

The reported duration of the effect of PKC activation on ion channel activity also varies considerably, with both readily reversible (< 5 min) and persistent responses (> 30 min) being documented. These responses do not appear to be related to the species of ion channel or the cell type in which it is studied (compare effects on I_K in cerebral hemisphere and hippocampal pyramidal neurones, and glial cells). The reversible response is likely to be explained by the rapid, sequential action of a kinase and a phosphoprotein phosphatase. The identity of the phosphatase(s) involved has not been determined, but effective dephosphorylation of the skeletal muscle DHP receptor, after prior phosphorylation by PKA or Ca^{2+}/calmodulin kinase, has been demonstrated biochemically, using calcineurin (90).

Explanation of the persistent response is more complicated, and as this phenomenon has not been investi-

gated in any detail, most of the explanations that can be advanced remain speculative. In some cases, the prolonged duration of the observed response could be related to a nonspecific membrane effect of the phorbol esters used, but where appropriate control experiments were performed using structurally related compounds showing no activity toward PKC, this can be discounted. Another possibility, by analogy with the reversible response, is that the transferred phosphate moiety is stable, either due to its inaccessibility, or to a concomitant inhibitory action of PKC on the phosphoprotein phosphatase. An alternative mechanism to be considered, is the temporal activation of the enzyme itself. Recent studies have shown that the level of enzyme activity, and also the relative expression of subspecies within a cell, can be influenced by certain activators, such as TPA (10). It is possible that while recording ion channel activity during prolonged application of phorbol esters, the cellular PKC activity changes from one of sustained activation to gradual inactivation. Circumstantial evidence for such a dual action of TPA on ion channel current, through changes in PKC activity, is provided by the results on Lacerda et al. (126). These authors have reported that short-term exposure (5 s) of cardiac ventricular cells to TPA results in an enhanced $I_{Ca(L)}$ whereas longer exposure (20 min) significantly reduces the conductance through this channel. Although this is still mainly a hypothetical situation, in only one electrophysiological study has the level of PKC activity been monitored before and after experimental manipulation. It remains, therefore, a possibility to be disproved or otherwise. In contrast to this line of thought, sustained activation of PKC, possibly as a result of a phospholipid-induced change in enzyme conformation, has been proposed to underlie the persistent inhibitory action of PKC on two species of K^+ channel in *Hermissenda* Type B photoreceptors (6). This model has yet to be substantiated on a firm biochemical basis, and its application to other cells remains unclear, but it explores a novel, interesting aspect of the potential mechanism of action of PKC, both for the modulation of ion channel function and other long-term cellular effects.

C. Modulation by PKC is Integrated with Effects of Other Second Messenger Systems

Direct activation of PKC with a DAG or phorbol ester does not always elicit the same overall effect on membrane conductance as an agonist that promotes activation of the enzyme by a receptor-mediated pathway. This indicates that the agonist may (*a*) also stimulate the production of other messenger molecules capable of influencing distinct channels, (*b*) influence the modulatory action observed with PKC alone as a result of (*a*), and (*c*) interact with other signal transduction pathways that can modulate ion channel activity in the same cell. The first two of these additional effects of agonists are illustrated by the response of NG108–15 cells to bradykinin (85) and the reported response of rat dorsal raphe neurones to α_1-adrenergic agonists (72). In the situation where an agonist stimulates the formation of more than one messenger molecule capable of altering ion channel activity, the relative efficacy of the messengers for modulating the different channels, and the relative density of the channels in the cell, are likely to be important factors in determining the overall change in membrane conductance.

The interaction of neurotransmitter receptors, through common components of the signal transduction machinery, to effect both convergent and divergent modulation of ion channels was highlighted in a recent review article by Nicoll (166). Many species of ion channel are now known to be coupled directly to receptors by *Pertussis* toxin-sensitive G-proteins, and in some cases (for example, DRG cells), this interaction has been reported to be affected following PKC activation. Immunohistochemical staining of rat brain tissue with a polyclonal antiserum selective for the α-subunit of G_0 has shown this protein to be abundant in the neuropil but absent from neuronal cell bodies (251). Areas such as the hippocampal formation and the molecular layers of the cerebral cortex and cerebellum, that are enriched in G_0 immunoreactivity, also express high levels of PKC, and it may be discovered that this colocalization has some functional basis for presynaptic ion channel modulation. It should be noted, however, that pharmacologically similar G-proteins can couple to phospholipase C, phospholipase A_2 and adenylate cyclase, and at present there is no clear indication that the observed modulatory action of PKC is specific for a G-protein/ion channel interaction, or is the result of an indirect effect on the G-protein 'pool' in the membrane.

In a number of cell types, PKC and PKA can interact at the membrane level to influence certain channel currents. In some cases, both protein kinases target the same species of channel ($\downarrow I_{K(Ca(S))}$, hippocampus; $\uparrow I_{Ca(L)}$, skeletal muscle), in other cases different channels ($\uparrow I_{Ca}$ versus $\downarrow I_K$, *Aplysia*). For channels that serve as a convergent substrate for both PKC and PKA, it has not yet been determined whether the dual modification of the channel results in an additive response, or if the action of one kinase overrides that of the other. This is an important area for future investigations, as little is known about why such interactive mechanisms exist, or how the actions of the different protein kinases are coordinated by the cell to control its response to various extracellular signals.

The primarily electrophysiological studies carried out in the last few years have yielded much new information concerning the modulation of ion channel activity by PKC. It is clear that this form of modulation is particularly diverse, and in this review we have attempted to present a broad perspective on the subject. Because of

their heterogeneity, progress in our understanding of the regulation of ion channels by protein kinases will depend critically on the discovery and development of specific agents that will allow qualitative pharmacological dissection of channel currents, and on the recording of single channel currents from the isolated channel populations. This may be facilitated by the use of excised membrane patches or by the use of a suitable expression system for a cloned channel gene. In addition, the use of purified channels, or recombinant (fusion?) channels, reconstituted into vesicles of defined lipid and protein content, is an approach that is likely to become more prominent.

Several studies have shown that injection or intracellular dialysis of preparations of purified PKC can affect ion channel currents. In all but one study, however, the enzyme was a mixture containing multiple subspecies, and so the contribution of each enzyme to the response could not be known. Although there is no direct evidence at present to propose a differential action of the subspecies on ion channel activity, the recent increased knowledge of their cellular distribution and catalytic properties suggests this as an increasingly attractive possibility and makes it an important direction of future studies.

Acknowledgments. The authors would like to thank Dr. Masaki Kameyama for his advice on electrophysiological recording techniques and, together with Dr. Ushio Kikkawa and Professor Haruhiro Higashida, for constructive comments on the manuscript. We are indebted to those colleagues who have given permission for us to cite unpublished work.

Work in the Department of Biochemistry, Kobe University School of Medicine, has been supported in part by research grants from the Scientific Research Fund of Ministry of Education, Science and Culture, Japan, Muscular Dystrophy Association, Yamanouchi Foundation for Research on Metabolic Disorders, Merck Sharp & Dohme Research Laboratories, Biotechnology Laboratories of Takeda Chemical Industries, Ajinomoto Central Research Laboratories, Meiji Institute of Health Sciences, Juvenile Diabetes Foundation International and New Lead Research Laboratories of Sankyo Company. M.S.S. is a recipient of a Research Fellowship from the Japan Society for the Promotion of Science.

Notes Added in Proof

Functional expression of a cloned rat hippocampal A-type K^+ channel protein (CHRISTIE, M. J., ADELMAN, J. P., DOUGLASS, J., AND NORTH, R. L. Science (Wash. DC) **244:** 221–224, 1989) and reconstitution of noxiustoxin affinity-purfied proteins from squid axons to form a K^+ channel resembling the delayed rectifier (PRESTIPINO, G., VALDIVIA, L. L., LIÉVANO, DARSZON, A., RAMÍREZ, A. N., AND POSSANI, L. D. FEBS Lett. **250:** 570–574, 1989) has now provided further evidence that the 70 kDa polypeptide encoded by the *Shaker* locus of *Drosophila*, and its 55 kDa counterpart(s) in mammalian brain (TEMPEL, B. L., JAN, Y. N., AND JAN, L. Y. Nature (Lond.) **332:** 837–839, 1988) is likely to be a principal component of many types of voltage-dependent K^+ channel.

The uncertainty regarding the mechanism of modulation in different cells of K^+ channels carrying the M-current has been prolonged further by two reports, in frog ganglion cells (BOSMA, M. M., AND HILLE, B. Proc. Natl., Acad. Sci. USA **86:** 2943–2947, 1989) and PC 12 cells (VILLARROEL, A., MARRION, N. V., LOPEZ, H., AND ADAMS, P. R. FEBS Lett. **255:** 42–46, 1989), wherein, although a partial reduction in $I_{K(M)}$ was observed following activation of PKC, the relevance of this effect to that evoked by agonist application has been questioned.

The outwardly rectifying Cl^- channels present in normal airway epithelial cells have been found to be a convergent substrate for both PKA and PKC (HWANG, T-C., LU, L., ZEITLIN, P. L., GRUENERT, D. C., HUGANIR, R. AND GUGGINO , W. B. Science (Wash. DC) **244:** 1351–1353, 1989; LI, M., MCCANN, J. D., ANDERSON, M. P., CLANCY, J. P., LIEDTKE, C. M., NAIRN, A. C., GREENGARD, P., AND WELSH, M. J. *ibid,* 1353–1356). Activation of these channels by phosphorylation has been found to be defective in cells from patients with cystic fibrosis, providing an explantation for the impaired production of respiratory tract fluid in this condition.

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