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Review

Protein kinase C regulation of $GABA_A$ receptors

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Abstract. Pharmacological studies with drugs that activate or inhibit several protein kinase C (PKC) isozymes have identified the PKC family of serine-threonine kinases as important in the regulation of γ-aminobutyric acid type A ($GABA_A$) receptor function. PKC modulates $GABA_A$ receptor surface density, chloride conductance and receptor sensitivity to positive allosteric modulators such as neurosteroids, ethanol, benzodiazepines and barbiturates. Recent studies using PKC isozyme-selective reagents and gene-targeted mice have begun to identify critical roles for three isozymes, PKCβII, PKCε and PKCγ, in various aspects of $GABA_A$ receptor regulation. Progress in this field touches upon therapeutic areas that are of great clinical importance such as anxiety and addiction. Increased understanding of how PKC regulates $GABA_A$ receptors and which PKC isozymes are involved holds promise for development of new treatments for diverse neuropsychiatric disorders.

Key words. Protein kinase C; gamma-aminobutyric acid; neurosteroid; ethanol; phosphorylation; receptor for activated C kinase; phorbol ester.

Introduction

γ-aminobutyric acid type A ($GABA_A$) receptors mediate the majority of fast inhibitory neurotransmission in the adult central nervous system [1, 2]. Binding of GABA to $GABA_A$ receptors opens an intrinsic anion channel that passes chloride ions. This usually hyperpolarizes the membrane, reducing the generation of action potentials. Mammalian $GABA_A$ receptors in the central nervous system are pentameric complexes composed of seven classes of subunits, derived from at least 18 different genes: α1–6, β1–3, γ1–3, δ, ε, θ and π [1–3]. In addition, splice variants identified for γ2, γ3, β3 and α6 add to this complexity. Most receptors in the mammalian brain are composed of α, β and γ subunits with α1β2γ2 being the most abundant combination [2].

$GABA_A$ receptors possess several modulatory sites that allosterically control activation by GABA. Mutagenesis and heterologous expression studies have identified binding sites and subunit combinations responsible for the pharmacological effects of several modulators. In addition, it has become increasingly clear that the actions of several drugs may be profoundly altered by phosphorylation. Several $GABA_A$ receptor subunits contain consensus sequences for phosphorylation by protein kinases, including Src family tyrosine kinases, PKA, PKC and Ca^{2+}-calmodulin-dependent kinase II [4]. Much evidence indicates that PKC plays a particularly important role in regulating $GABA_A$ receptor trafficking, response to GABA and response to allosteric modulators. Some of the strongest evidence for this has come from studies using gene knockouts that have identified effects of specific PKC isozymes on $GABA_A$ receptor pharmacology [5–8]. Here we review current knowledge of $GABA_A$ receptor regulation by PKC.

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PKC diversity and regulation

PKC is a family of phospholipid-dependent serine-threonine kinases that transduce signals involving lipid second messengers [9, 10]. Nine PKC genes have been identified. The first that were discovered comprise the 'conventional' cPKC subgroup (α, β and γ), which encodes enzymes activated by calcium, phosphatidylserine (PS) and diacylglycerol (DAG). Low stringency hybridization of complementary DNA (cDNA) libraries led to subsequent cloning of 'novel' nPKCs (δ, ε, η and θ) which are activated by DAG and PS but not by calcium, and 'atypical' aPKC isozymes (ζ and λ/ι) which are insensitive to calcium and DAG, but are activated by other lipid messengers. PKCλ and PKCι are the respective human and mouse homologues of the same enzyme. The PKCβ gene was found to generate two alternative splice variants [11]; splice variants have also been described for PKCδ [12] and PKCθ [13]. There also exists a short form of PKCζ, termed PKMζ, that is transcribed from an internal promoter of the PKCζ gene [14].

Additional kinases, related to the PKC family are the PKC-related kinases PRK1 and PRK2, which are insensitive to calcium and DAG but show increased activity when bound to activated RhoA GTPase [15]. Two other enzymes, referred to as PKCμ and PKCν, are activated by DAG but not by calcium; they contain novel functional domains and display a pattern of substrate specificity in vitro that differs from PKC isozymes, which has led to their recent designation as members of the PKD family [16].

Several lipid second messengers stimulate PKC isozymes. The first described pathway involves phosphoinositide (PI) signaling. Activation of cell surface receptors coupled to phospholipase C (PLC) stimulates hydrolysis of phosphatidylinositol-4,5-bisphosphate to generate inositol triphosphate (IP_3) and DAG. IP_3 binds to intracellular receptors, thereby stimulating release of calcium from intracellular stores, while DAG activates conventional and novel PKC isozymes. Receptor-mediated activation of phospholipase D also produces DAG, whereas *cis*-unsaturated fatty acids, arachidonic acid and lysophosphatidycholine produced by phospholipase A_2 can activate or enhance activation of several PKC isozymes [10, 17, 18]. Finally, phosphatidylinositol-4,5-bisphosphate and phosphatidylinositol-3,4,5-triphosphate, produced by receptor-mediated activation of phosphoinositide 3-OH kinase (PI 3-kinase), activate several novel PKC isozymes in vitro [19].

Sequential phosphorylation at two or three sites in the kinase domain of PKCs is required for maximal PKC activity. The first and rate-limiting step occurs in the activation loop and is catalyzed by phosphoinositide-dependent kinase 1 (PDK1), which is regulated by 3′ phosphoinositides generated by PI 3-kinase [20]. Phosphorylation of this residue correctly aligns the active site, conferring a low level of catalytic activity that permits autophosphoylation of one or two additional residues at the C-terminus. This results in a mature enzyme capable of responding to lipid second messengers. PDK1 phosphorylation of the activation loop is a constitutive event in the processing and maturation of newly translated cPKCs, whereas for nPKCs and aPKCs, phosphorylation by PDK1 is under partial regulation by PI 3-kinase [20, 21]. PKC function is also regulated by localization; activation is generally associated with translocation of PKC from one cellular compartment to another containing lipid activators and isozyme-specific anchoring proteins that bind the activated form of the enzyme in proximity to substrates [22].

Tumor-promoting phorbol esters bind to PKCs at the DAG site in the regulatory domain and stimulate prolonged activation of cPKCs and nPKCs [23]. They have been used extensively as pharmacological probes of PKC function. It is important to note that phorbol esters bind and modulate the function of other molecules besides PKC [24], and therefore responses to phorbol esters should not be assumed to be due to PKC activation without corroborating evidence. One approach is to reverse phorbol ester effects with PKC inhibitors that act at the ATP binding site of the kinase.

Several compounds have been identified that inhibit PKC activity [25]. Though these compounds exhibit different affinities for PKC isozymes in vitro, very few are isozyme specific. One compound, Gö 6976, is a selective inhibitor of cPKCs [26] and PKD1 (PKCμ) [16], whereas LY333531 [27] selectively inhibits PKCβ when compared with other PKC isozymes. Based on sequence analysis of domains that bind isozyme-specific anchoring proteins, Mochly-Rosen and colleagues [28, 29] have identified peptide inhibitors and activators of cPKCs, PKCε and PKCδ. These reagents have provided researchers with some of the most selective probes of PKC function to date. Finally, several PKC isozymes have been deleted by gene targeting in mice [30], and studies with these mice have been especially useful for analysis of PKC function in vivo.

PKC regulation of GABA-stimulated chloride currents

Several laboratories have reported that phorbol esters reduce $GABA_A$ receptor activation by GABA or by the alkaloid muscimol, which acts as a direct receptor agonist at the GABA binding site [31–34]. In studies with recombinant expression systems, this effect has been found using $\alpha1\beta1$, $\alpha1\beta1\gamma2S$ or $\alpha1\beta1\gamma2L$ [32], $\alpha5\beta2\gamma2S$ or $\alpha5\beta2\gamma2L$ [34], and $\alpha1\beta2\gamma2S$ [31] receptors. The most striking finding is a reduction in current amplitude with

little change in kinetics, occurring 2–20 min after application of phorbol ester. Functional studies using site-directed mutagenesis determined that this down-regulation of receptor function is mediated by phosphorylation of β1 S409 [32], β2 S410 [31], γ2S S327 [31, 32], and γ2L S327 and S343 [32]. Moreover, these same sites, as well as S408 and S409 in β3, can be phosphorylated by PKC in vitro [35, 36]. These findings suggest that PKC can phosphorylate β and γ2 subunits to decrease activation of $GABA_A$ receptors by GABA.

In contrast to studies using phorbol esters, different results were reported using an active catalytic domain of PKC (PKM), which enhanced GABA-stimulated Cl^- currents when perfused intracellularly into mouse L929 fibroblasts [37, 38] or rat hippocampal dentate granule cells [39]. In transfected fibroblasts, PKM increased the maximal response to GABA, slightly increased the half-maximal effective concentration, but did not alter channel kinetics [37]. In dentate granule cells, PKM increased the peak amplitude of miniature inhibitory postsynaptic currents [39]. Transfection of L929 mouse fibroblasts with α1β1γ2L subunits containing mutations S409A in β1, or S327A and S343A in γ2L inhibited enhancement of $GABA_A$ currents by PKM [37].

A major concern in these studies is the fact that PKM lacks key regulatory sequences important for targeting of individual PKC isozymes to different subcellular locations upon activation [22]. Therefore, PKM could mimic the actions of multiple PKC isozymes, or, alternatively, PKM might exhibit completely promiscuous actions unrelated to normal PKC function. However, treatment of hippocampal neurons with brain-derived neurotrophic factor (BDNF) transiently enhances $GABA_A$ currents, and this correlates in time with association of PKC with β3 subunits and phosphorylation of β3 at S408/409 [40]. Although the PKC isozyme involved was not identified, this study nevertheless suggests that activation of an endogenous PKC can enhance $GABA_A$ receptor function.

Since these studies used approaches that do not manipulate individual PKC isozymes, it is perhaps not surprising that they provided conflicting results with regard to the direction in which $GABA_A$ receptors are regulated by PKC. One possible explanation may be that these different approaches detect effects of different PKC isozymes. It is striking that in these studies, site-directed mutagenesis of the same phosphorylation sites in β1 and γ2 subunits decreased phorbol ester-induced inhibition and PKM-induced enhancement of $GABA_A$ receptor function. This may indicate that additional PKC substrates besides β and γ2 subunits are phosphorylated by specific PKC isozymes to set the direction of the response towards inhibition or enhancement of $GABA_A$ receptor function.

It is not known which PKC isozymes mediate phorbol ester-induced alterations in $GABA_A$ receptor function, though current evidence implicates conventional PKC isozymes. In HEK-293 cells, overexpression of PKCα was used to observe phorbol ester-mediated down-regulation of GABA-stimulated currents, suggesting that PKCα can support this response [32]. Other evidence implicates PKCβII, which with its anchoring protein RACK1 binds to the major intracellular domain of β subunits in HEK293 cells and in adult rat brain [41, 42] (fig. 1). In neurons from rat prefrontal cortex, 5-HT_2 agonists reduce postsynaptic $GABA_A$ currents [43], and this is prevented by a PKC pseudosubstrate inhibitor peptide or by a peptide [44] that prevents binding of RACK1 to PKCβII. Moreover, in HEK293 cells expressing α1β1 subunits, mutation of the serine residue at position 409 to alanine in β1 [32], or application of a peptide that inhibits RACK1 binding to β1 [42], reduces phorbol ester-mediated down-regulation of $GABA_A$ receptor function. This peptide also reduces down-regulation of $GABA_A$ currents in superior cervical ganglion cells treated with phorbol ester or with muscarine. These cells express β1-containing $GABA_A$ receptors and M1 muscarinic acetylcholine receptors coupled to PLC [42]. Taken together, these results suggest that activated PKCβII down-regulates $GABA_A$ receptor function by phosphorylating S409 in β1 subunits. Since PKC also phosphorylates serines 408 and 409 in β3 subunits [35], PKCβII and RACK1 bind β3 subunits [41] and incubation of cortical neurons with phorbol-12-myristate-13-acetate (PMA) [41] or mus-

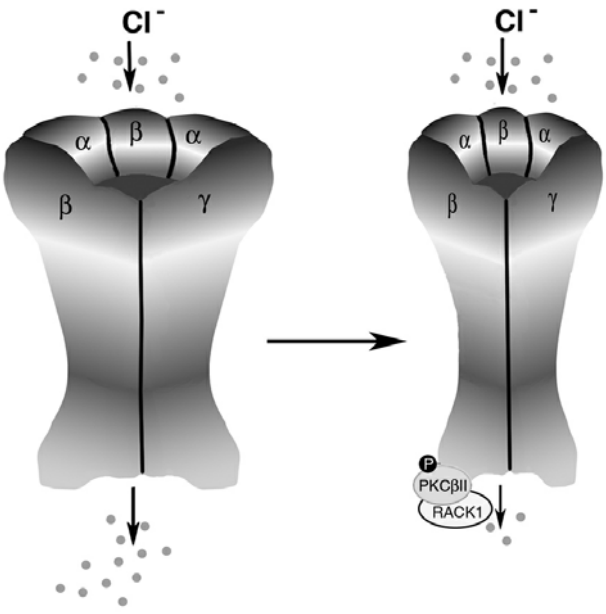

Figure 1. Downregulation of $GABA_A$ receptor function by PKC. Treatment with phorbol esters decreases $GABA_A$ current independent of changes in receptor cell surface expression. Likely substrates mediating this effect are β subunits, which are phosphorylated by PKC at S409 in β1, S410 in β2 and S408/409 in β3. RACK1 and PKCβII bind β subunits in response to phorbol esters, and this correlates with β subunit phosphorylation, making PKCβII the likely mediator of this response.

carine [42] stimulates PKC-dependent phosphorylation of β3 subunits, PKCβII may also modulate $GABA_A$ receptors through phosphorylation of β3-containing receptors. However, in these cases PKC might be expected to increase receptor function since PKA-mediated phosphorylation of S408/409 in β3 enhances GABA-stimulated currents, in contrast to PKA phosphorylation of S409 in β1, which inhibits $GABA_A$ receptor function [45]. Proof of a major role for PKCβII in modulation of $GABA_A$ receptor function awaits studies in which this PKC isozyme is selectively inhibited by isozyme-selective strategies such as gene targeting, RNA interference or treatment with selective inhibitors such as LY333531.

PKC regulation of $GABA_A$ receptor trafficking

$GABA_A$ receptors undergo constitutive recycling between the cell surface and endosomes; in neurons and in HEK293 cells, this involves a clathrin-dependent pathway [46, 47], whereas in HEK293 cells, clathrin-independent endocytosis of $GABA_A$ receptors has also been documented [48]. In cultured hippocampal neurons $GABA_A$ receptors are co-localized in the plasma membrane with β2-adaptin in AP2 complexes, and AP2 complexes from brain membrane extracts will bind to glutathione S-transferase-conjugated intracellular domains of $GABA_A$ receptor β*1,* β*3* and γ2 subunits [46]. A dileucine motif in the intracellular loop of $GABA_A$ receptor β subunits appears to be important for interaction with AP2 complexes and for clathrin-dependent endocytosis of $GABA_A$ receptors [49]. It is not known if this occurs through binding of the dileucine motif to β2-adaptin of the AP2 complex.

In recombinant systems such as HEK-293 cells or *Xenopus* oocytes, phorbol esters decrease the amplitude of GABA-induced chloride currents, in part, by inducing down-regulation of cell surface $GABA_A$ receptors through a process independent of PKC phosphorylation at sites identified in β1–3 or γ2 subunits [50–52]. PKC inhibitors block this effect of phorbol esters, without altering basal levels of cell surface receptors [48, 50, 51]. There is some controversy as to the mechanism by which phorbol esters alter cell surface stability of $GABA_A$ receptors in heterologous expression systems. Several investigators have interpreted their results as suggesting that phorbol esters stimulate $GABA_A$ receptor internalization [48, 50, 52]. However, Connolly and colleagues [51] found evidence to suggest that phorbol esters reduce surface levels of $GABA_A$ receptors by preventing recycling of receptors to the cell surface. The major evidence for this was that wortmannin, which inhibits receptor recycling to the cell membrane, also reduced surface levels of $GABA_A$ receptors and combined treatment with wortmanin and PMA did not result in further reduction of surface expression. These mechanisms, however, may not be mutually exclusive, and could involve different PKC isozymes. Known PKC substrates that might play a role in this process include dynamin, which associates with AP2 complexes and is released from the cell membrane to the cytosol in response to PKC phosphorylation [53, 54], and the N-ethylmaleimide-sensitive factor (NSF), which plays an important role in vesicle fusion during exocytosis and recycling (fig. 2).

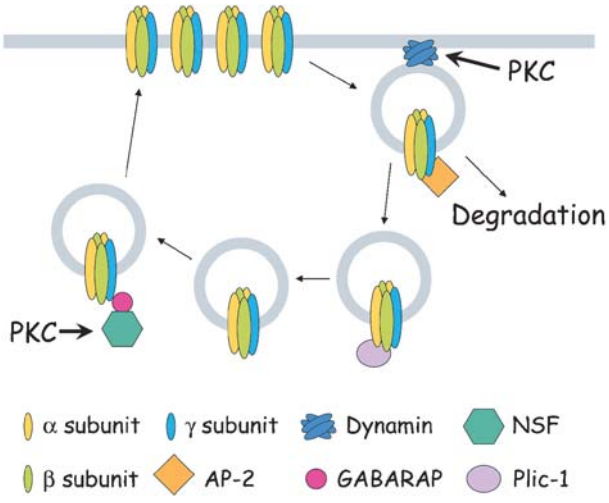

Figure 2. $GABA_A$ receptor trafficking. Intracelluar domains of β or γ subunits bind AP-2 complexes, which recruit clathrin. Clathrin-coated vesicles are released from the membrane by dynamin. Endocytosed receptors are degraded, or are protected from degradation by interaction of intracellular domains of α or β subunits with Plic-1, which stabilizes the intracellular pool of $GABA_A$ receptors. GABARAP in complex with NSF binds to the intracellular domain of γ2 subunits and delivers the receptors to the membrane from the Golgi apparatus and from endosomes. Membrane-bound dynamin is phosphorylated by PKC, allowing it to relocate to the cytosol for the next round of endocytosis. When NSF is phosphorylated by PKC it becomes unable to bind SNAP-SNARE complexes. Activation of PKC may reduce the surface expression of $GABA_A$ receptors through dynamin or NSF.

It is not clear whether PKC activation produces down-regulation of $GABA_A$ receptor cell surface expression in neurons. In cultured rat cerebellar granule cells, phorbol esters decrease cell surface expression of $GABA_A$ receptors, but it is not known if this is due to activation of PKC since the effect of PKC inhibitors was not tested [55]. On the other hand, phorbol esters induce down-regulation of $GABA_A$ currents without decreasing the number of $GABA_A$ receptors on the cell surface in cultured rat cortical neurons [56]. In the mouse superior colliculus, BDNF decreases $GABA_A$ receptor current amplitudes, whereas deletion of BDNF through gene inactivation or inhibition of TrkB increases $GABA_A$ receptor current amplitudes via a PKC-dependent mechanism [57]. Whether this results from BDNF regulation of receptor density or channel conductance is not yet known. Brunig and colleagues [58] found that in cultured hippocampal neurons

BDNF produces a down-regulation of $GABA_A$ receptor function that is associated with a decrease in cell surface receptor expression. However, Javanovic and colleagues [40] more recently reported a more complex response to BDNF in rat hippocampal neurons. They found that BDNF induces a transient recruitment of surface $GABA_A$ receptors and an increase in receptor function, followed by sustained down-regulation of function that is not associated with loss of cell surface receptors. These responses were associated with time-correlated increases and then decreases in phosphorylation of β3 subunits at S408/9 and appeared to be PKC-dependent since they could be blocked by calphostin C. Thus, while it is clear that PKC participates in BDNF regulation of $GABA_A$ receptor function, it is not certain that this involves changes in receptor density. In summary, although there is good evidence for PKC-induced down-regulation of $GABA_A$ receptor function in neurons, the best evidence at present supports an effect on channel gating or conductance rather than on receptor trafficking.

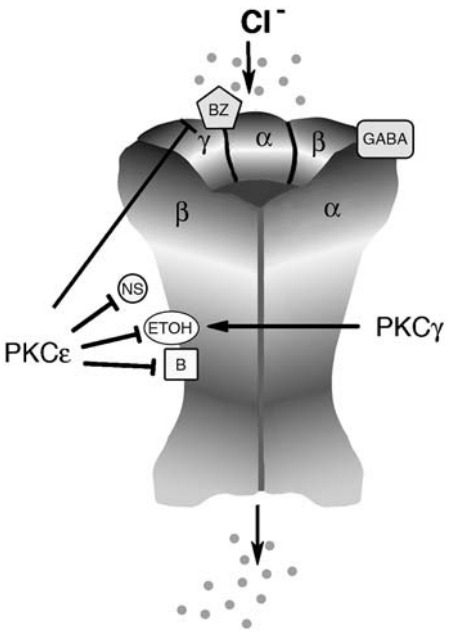

Figure 3. PKC modulation of allosteric sensitivity. Shown is a schematic diagram of postulated binding sites or receptor domains important for interaction of GABA, benzodiazepines (BZ), ethanol (ETOH) and barbiturates (B) with $GABA_A$ receptors [88, 89]. Binding sites for neurosteroids (NS) have not been identified and may involve other proteins besides receptor subunits [86]. Based on findings in gene-targeted mice, PKCγ facilitates ethanol actions at $GABA_A$ receptors, whereas PKCε diminishes the allosteric actions of benzodiazepines, barbiturates, neurosteroids and ethanol.

PKC regulation of $GABA_A$ receptor sensitivity to allosteric modulators

Several studies have implicated PKC in modulation of $GABA_A$ receptors by ethanol. Intoxicating concentrations of ethanol enhance $GABA_A$ receptor function in several neuronal preparations [59–61]. Early work suggested that ethanol increases $GABA_A$ receptor function in only certain brain regions, such as the frontal cortex, medial septum and inferior colliculus, but not in the lateral septum or the hippocampus [62–64]. Later work demonstrated that ethanol increases hippocampal $GABA_A$ receptor function under certain circumstances. For example, addition of intracellular ATP during whole cell patch clamp recording [65], cold (11–15 °C) shock treatment of hippocampal slices [66] or inhibition of $GABA_B$ receptors [67] can unmask ethanol enhancement of $GABA_A$ currents. In addition, ethanol, unlike other allosteric $GABA_A$ receptor modulators, enhances only proximal but not distal $GABA_A$ inhibitory postsynaptic potentials in hippocampal CA1 neurons [68]. PKC inhibitors suppress enhancement of $GABA_A$ receptor function by ethanol in hippocampal neurons, suggesting that PKC-mediated phosphorylation is required for ethanol sensitivity in those cells [66, 68].

Early in vitro expression studies using *Xenopus* oocytes or transfected mouse L(tk-) cells suggested a requirement for the long splice variant of γ2 subunits, γ2L, in conferring PKC-mediated sensitivity to low doses (< 50 mM) of ethanol [69–72]. In transfected mouse L(tk-) cells, this requirement was detected by $^{36}Cl^-$ flux measurements, but was not observed for receptors expressed in *Xenopus* oocytes and examined using electrophysiological techniques [69]. Others have failed to observe a requirement for γ2L in conferring sensitivity to low concentrations of ethanol [73-75], and mice lacking γ2L show normal behavioral and electrophysiological responses to ethanol [76]. Thus, it is unlikely that splice variation in γ2 determines ethanol sensitivity.

More recent studies have used knockout mice to examine the roles of individual PKC isozymes in regulating $GABA_A$ receptor function and have identified two, PKCγ and PKCε, that differentially modulate actions of ethanol at these receptors (fig. 3). PKCγ null mice show reduced sensitivity to the acute effects of ethanol on loss of the righting reflex and body temperature, but have normal responses to pentobarbital and flunitrazepam [5, 77]. This is associated with decreased in vitro sensitivity of $GABA_A$ receptors to ethanol, but not to muscimol, pentobarbital or flunitrazepam. PKCγ null mice also self-administer more ethanol than wild-type littermates [78].

In contrast, mice that lack PKCε consume 50–75% less alcohol and show markedly reduced alcohol preference, operant self-administration of ethanol and relapse drinking following alcohol deprivation. These mice also show heightened behavioral sensitivity to the acute locomotor activating effects of low doses of ethanol as well as to the hypnotic effects of higher ethanol doses, when compared

with wild-type littermates [7, 79]. These behaviors are associated with increased enhancement of $GABA_A$ receptor function by ethanol in microsacs from the frontal cortex of PKCε null mice [7, 8]. Application of a peptide that selectively inhibits PKCε [80] increases ethanol modulation of $GABA_A$ receptors in synaptosomes from wild-type mice but not from PKCε null mice. In addition, restoration of PKCε by means of tetracycline-regulated transgenic expression elevates ethanol intake and reduces acute behavioral responses to ethanol to levels observed in wild-type mice. These findings indicate that increased ethanol sensitivity, reduced ethanol consumption and enhanced ethanol modulation of $GABA_A$ receptors in PKCε null mice are due to loss of PKCε in adult neurons and not to changes resulting from absence of PKCε during development. Thus, it appears that at least two members of the PKC family modulate $GABA_A$ receptor sensitivity to ethanol, but their actions are antagonistic to each other. These observations underscore the importance of strategies that selectively manipulate individual PKC isozymes to understand the function of different PKC family members.

Besides ethanol, PKC modulates sensitivity of $GABA_A$ receptors to other positive allosteric modulators. Several studies have suggested that activating PKC increases $GABA_A$ receptor responses to these drugs. Using *Xenopus* oocytes that express recombinant $GABA_A$ receptors composed of $\alpha 1\beta 1\gamma 2$L subunits, Leidenheimer and colleagues found that PMA increases allosteric actions of diazepam, pentobarbital [81] and allopregnanolone [82] on GABA-induced chloride currents. These effects were inhibited by staurosporine, a broad-spectrum kinase inhibitor that inhibits several PKC isozymes and other kinases [25]. However, Ghansah and Weiss [83] were unable to confirm results for pentobarbital or diazepam using the same expression system and receptor subunits. Fancsik and colleagues [84] found that in oxcytocin-producing magnocellular neurons of the male rat supraoptic nucleus, neurosteroid potentiation of GABA-induced current is inhibited by bisindolylmaleimide I, suggesting a facilitatory role for PKC in this response. However, treatment with PMA did not promote enhancement by allopregnanolone, raising the possibility that the effect of bisindolylmaleimide I was not due to inhibition of PKC. More recently, Harney and colleagues [85] found that response of synaptic $GABA_A$ receptors to allopregnanolone could be reduced in CA1 neurons by inhibiting PKC with a pseudosubstrate peptide and could be enhanced in dentate granule cells by phorbol ester. Unfortunately, the effect of phorbol ester in dentate granule cells was not challenged with a PKC inhibitor, and in CA1 neurons phorbol ester had no effect, raising some uncertainty as to whether these events are mediated by PKC. Nevertheless, these studies suggest that in some cells, activation of PKC promotes the sensitivity of $GABA_A$ receptors to positive allosteric modulators. The PKC isozymes that mediate this response are not known.

Other studies suggest that activation of PKC can reduce the actions of allosteric modulators on $GABA_A$ receptors. In rat cortical neurons, PKC inhibition increases the ability of allopregnanolone to prolong GABA miniature inhibitory postsynaptic currents [86]. Recently, Koksma and colleagues [87] elegantly demonstrated the importance of PKC in mediating the decline in neurosteroid sensitivity of magnocellular supraoptic neurons that occurs after parturition in female mice. This change in neurosteroid sensitivity leads to increased firing of supraoptic neurons and the timed release of oxytocin. They found that during late pregnancy, when $GABA_A$ receptors are neurosteroid-sensitive, treatment with inhibitors of protein phosphatase 1 and 2A or with PMA suppresses neurosteroid modulation of $GABA_A$ receptors. Conversely, after parturition, when $GABA_A$ receptors become neurosteroid-resistant, sensitivity to neurosteroid modulation can be restored by activating phosphatases or by inhibiting PKC with chelerythrine.

Studies with PKCε null mice suggest that PKCε inhibits modulation of $GABA_A$ receptors by positive allosteric activators. In addition to altered responses to ethanol (see above), these mice show increased sensitivity to the acute behavioral effects of barbiturates, benzodiazepines and neurosteroids, and this is associated with increased in vitro sensitivity of $GABA_A$ receptors to these allosteric modulators [7, 8]. Consistent with the known anxiolytic properties of neurosteroids, PKCε null mice also show reduced anxiety-like behavior and reduced plasma levels of stress hormones [8]. Therefore, considering these findings, it is the likely that PKCε is the PKC isozyme responsible for reducing sensitivity of $GABA_A$ receptors to neurosteroids and other positive allosteric modulators in central neurons.

Conclusion

Many studies have implicated phorbol ester-sensitive PKC isozymes in the regulation of $GABA_A$ receptor chloride conductance, surface density and modulation by allosteric activators. PKC regulation of channel conductance appears to result from phosphorylation of β and possibly $\gamma 2$ subunits. However, different laboratories have provided conflicting results as to the direction of change induced by such phosphorylation. It is proposed that this might reflect opposing actions of different PKC isozymes or the phosphorylation of different β subunits. The current evidence indicates that PKCβII is the PKC isozyme most likely to mediate PKC-induced down-regulation of $GABA_A$ receptor function. PKC regulation of $GABA_A$ receptor surface density has been well documented in heterologous systems, but it is not clear if it

commonly occurs in neurons, or if it results from PKC-stimulated endocytosis or inhibition of receptor recycling to the cell surface. In addition, the PKC substrates are currently unknown, as are the PKC isozymes responsible for this modulation. Finally, studies with mice lacking PKCγ or PKCε have determined that these isozymes modulate the response of $GABA_A$ receptors to allosteric modulators. PKCγ enhances sensitivity to ethanol, whereas PKCε appears to diminish modulation by a variety of agents, including ethanol, barbiturates, benzodiazepines and neurosteroids. PKCε regulation of neurosteroid sensitivity may contribute to reduced anxiety-like behavior, supersensitivity to ethanol and diminished ethanol self-administration in PKCε null mice. These findings suggest that inhibitors of PKCε, through modulation of $GABA_A$ receptor function, may prove useful in the treatment of anxiety disorders and alcoholism.

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