Molecular Neurobiology

ISSN0893-7648/98/17(1–3): 1–15/$11.75

Neuronal Signaling Systems and Ethanol Dependence

Subhash C. Pandey

The Psychiatric Institute, Department of Psychiatry, College of Medicine, University of Illinois at Chicago and Psychiatry Research Service, VA Chicago Health Care System (West Side Division), Chicago, IL 60612

Abstract

In recent years there have been remarkable developments toward the understanding of the molecular and/or cellular changes in the neuronal second-messenger pathways during ethanol dependence. In general, it is believed that the cyclic adenosine 3′, 5′-monophosphate (cAMP) and the phosphoinositide (PI) signal-transduction pathways may be the intracellular targets that mediate the action of ethanol and ultimately contribute to the molecular events involved in the development of ethanol tolerance and dependence. Several laboratories have demonstrated that acute ethanol exposure increases, whereas protracted ethanol exposure decreases, agonist-stimulated adenylate cyclase activity in a variety of cell systems, including the rodent brain. Recent studies indicate that various postreceptor events of the cAMP signal transduction cascade (i.e., G_s protein, protein kinase A [PKA], and cAMP-responsive element binding protein [CREB]) in the rodent brain are also modulated by chronic ethanol exposure. The PI signal-transduction cascade represents another important second-messenger system that is modulated by both acute and chronic ethanol exposure in a variety of cell systems. It has been shown that protracted ethanol exposure significantly decreases phospholipase C (PLC) activity in the cerebral cortex of mice and rats. The decreased PLC activity during chronic ethanol exposure may be caused by a decrease in the protein levels of the PLC-β_1 isozyme but not of PLC-δ_1 or PLC-γ_1 isozymes in the rat cerebral cortex. Protein kinase C (PKC), which is a key step in the PI-signaling cascade, has been shown to be altered in a variety of cell systems by acute or chronic ethanol exposure. It appears from the literature that PKC plays an important role in the modulation of the function of various neurotransmitter receptors (e.g., γ-aminobutyrate type A [$GABA_A$], N-methyl-D-aspartate [NMDA], $serotonin_{2A}$ [5-HT_{2A}], and 5-HT_{2C}, and muscarinic [m_1] receptors) resulting from ethanol exposure. The findings described in this review article indicate that neuronal-signaling proteins represent a molecular locus for the action of ethanol and are possibly involved in the neuroadaptational mechanisms to protracted ethanol exposure. These findings support the notion that alterations in the cAMP and the PI-signaling cascades during chronic ethanol exposure could be the critical molecular events associated with the development of ethanol dependence.

Index Entries: cAMP-dependent protein kinase A; adenylate cyclase; cAMP-responsive-element binding protein; neurotransmitter receptor; protein kinase C; phospholipase C; second messengers; phosphoinositide signaling; ethanol dependence; ethanol tolerance.

Introduction

The phenomenon of ethanol dependence in humans or in animal models has been defined by the presence of ethanol withdrawal symptoms after the cessation of long-term ethanol intake (Tabakoff et al., 1986; Harris and Buck, 1990; Tabakoff and Rothstein, 1983). The intake of ethanol by alcoholics relieves ethanol withdrawal signs and symptoms (Harris and Buck, 1990; Tabakoff and Rothstein, 1983). The acute physiological effects of ethanol are reversible; after protracted ethanol intake, however, adaptive changes take place in the brain that alter the responsiveness of many of the molecular and cellular processes affected by acute ethanol intake.

Adaptation to protracted ethanol exposure is a multifaceted response that can be identified at different levels of complexity, ranging from alterations in the receptor protein, the effector enzymes, and the physical properties of membranes, to postreceptor events and gene expression in the brain (Goldstein, 1976; Hoffman and Tabakoff, 1990; Coe et al., 1996; Alling et al., 1994). In the past, the action of ethanol was considered to be mediated by the alteration in membrane fluidity caused by the partitioning of ethanol into neuronal-membranal lipids (Goldstein, 1976; Sun and Sun, 1985). Recent investigations have focused on the interactions between ethanol and specific neuronal-signaling proteins (Hoffman and Tabakoff, 1990; Coe et al., 1996; Alling et al., 1994; Tabakoff and Hoffman, 1996; Wand and Levine, 1991). Recent studies by several investigators indicate that changes in expression and function of certain neuronal proteins involved in signal transduction play an important role in the process of ethanol dependence (Goldstein, 1976; Hoffman and Tabakoff, 1990; Coe et al., 1996; Tabakoff and Hoffman, 1996; Wand and Levine, 1991). Molecular and/or cellular changes in the signaling cascade in the brain may be the basis of ethanol dependence, since these neuroadaptive changes occur as a consequence of the chronic ethanol exposure. This article will briefly review recent investigations of the molecular mechanisms involved in the changes in the cAMP-adenylate cyclase and the phosphoinositide (PI)-signaling cascades in the brain during ethanol dependence.

The cAMP Signaling System: Acute and Chronic Effects of Ethanol

The cAMP-signaling cascade (Fig. 1) consists of neurotransmitter receptors, guanine nucleotide-binding protein (G protein); adenylate cyclase; and cAMP-dependent protein kinase A (PKA). The enzyme adenylate cyclase is under the control of two kinds of G proteins, i.e., stimulatory (G_s) and inhibitory (G_i). G proteins are heterotrimers, consisting of α, β, and γ subunits. The catalytic unit of adenylate cyclase is activated or inhibited by $G_s\alpha$ or $G_i\alpha$, respectively (Neer, 1995; Gilman, 1989). Theoretically, the actions of various neurotransmitters (serotonin, norepinephrine, dopamine, and acetylcholine) in the brain are mediated through the activation or inhibition of adenylate cyclase. Adaptations in several steps of the cAMP signal-transduction pathway in the brain may play an important role in ethanol tolerance and dependence (Tabakoff and Rothstein, 1983; Hoffman and Tabakoff, 1990; Wand and Levine, 1991). In general, acute ethanol exposure in vitro or in vivo potentiates the receptor-mediated stimulation of adenylate cyclase activity, whereas chronic ethanol decreases the agonist-stimulated adenylate cyclase activity in neuronal membranes (Saito et al., 1985; 1987; Rabin, 1993; Bode and Molinoff, 1988).

Several previous studies have shown that agonist-stimulated (guanine nucleotides and β-adrenergic receptor agonists) adenylate cyclase activity is diminished in various brain structures of mice or rats treated chronically with ethanol (Hoffman and Tabakoff, 1990; Bode and Molinoff, 1988; Wand et al., 1991). It has also been suggested that chronic ethanol treatment results in the loss of the high-affinity

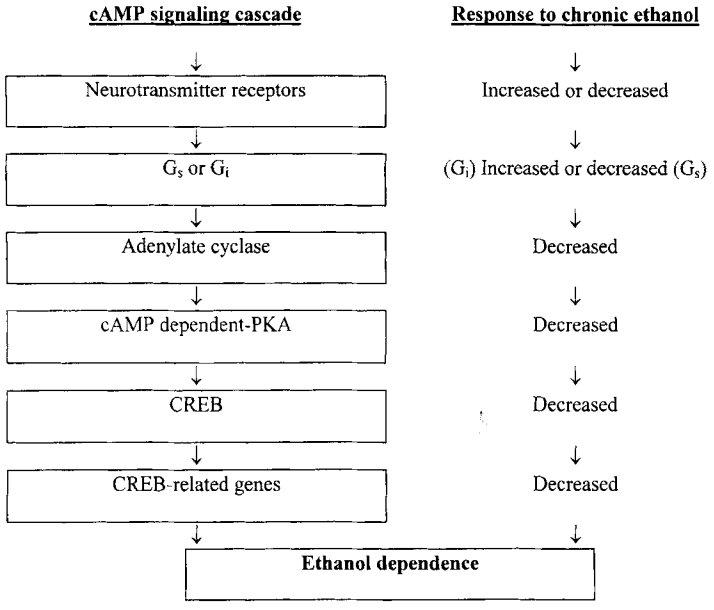

Fig. 1. The cAMP-signal transduction pathway in the brain and its responses to chronic ethanol exposure. G_s = stimulatory GTP-binding protein; G_i = inhibitory GTP-binding protein; PKA = protein kinase A; CREB = cAMP-responsive-element binding protein.

β-adrenergic-receptor agonist-binding site in the frontal cortex and the hippocampus, but not in the cerebellum of mice (Saito et al., 1987; Valverius et al., 1989a). Furthermore, complexes of $G_s\alpha$ and adenylate cyclase as measured by [^{3}H] forskolin binding, were decreased in various brain structures of mice by protracted ethanol exposure (Valverius et al., 1989b). Chronic exposure of cells to ethanol in medium also diminishes the activity of adenylate cyclase stimulated by receptor agonists and guanine nucleotides (Robin, 1990; Gordon et al., 1986; Mckenzie and Milligan, 1990). These results suggest that changes in adenylate cyclase activity and in cAMP content in the brain during chronic ethanol exposure may be the mechanism associated with the process of ethanol dependence.

The adenylate cyclase signal-transduction pathway has been proposed as a potential biological marker for alcoholism (Lichtenberg-Kraag et al., 1995; Nagy et al., 1988; Diamond et al., 1987). Decreased receptor-mediated activity of adenylate cyclase has been shown in platelets and lymphocytes of alcoholics (Nagy et al., 1988; Diamond et al., 1987; Tabakoff et al., 1988; Waltman et al., 1993). In fact, Wand et al. (1994) demonstrated that enhanced expression of $G_s\alpha$ may be a marker of increased risk for the future development of alcoholism. The decreased activity of adenylate cyclase may be caused by changes in the receptor or to changes in expression and function of G proteins during ethanol exposure. Waltman et al. (1993) reported that the expression of inhibitory $G_{i2}\alpha$ protein is increased, whereas the activity of adenylate cyclase is decreased in lymphocytes of abstinent alcoholics. Wand and Levine (1991) studied the expression of G_s and G_i protein and the activity of adenylate cyclase in the pituitary and the cerebellum during ethanol exposure of Long Sleep (LS) and Short Sleep (SS) mice. They observed decreased protein levels of G_s but not G_i, as well as decreased agonist-stimulated adenylate cyclase activity in these brain structures of LS mice chronically

treated with ethanol. On the other hand, protein levels of G_s and G_i, and adenylate cyclase activity were not changed in the pituitary and the cerebellum of SS mice treated with ethanol. It has been shown recently that adenylate cyclase activity is decreased during chronic ethanol exposure without accompanying changes in the levels of G_s and G_i proteins in the cerebral cortex of mice (Tabakoff et al., 1995). These authors studied forskolin- and manganese-stimulated adenylate cyclase activity in the cortex of ethanol-treated mice, and they observed decreased adenylate cyclase activity. These results suggest that ethanol may directly alter the activity of the catalytic unit of adenylate cyclase. The differences between the results of this study and the study of Wand and Levine (1991) could be related to the use of different brain regions and different treatment paradigms. Furthermore, Wand and Levine (1991) used a selected line of mice. Nonetheless, all these studies support the idea that cAMP-signal transduction may be an important intracellular target for the cellular adaptation to chronic ethanol exposure.

When animals are chronically exposed to ethanol, in most studies, their blood ethanol levels range from 100 to 400 mg%, whereas the ethanol concentration in most in vitro studies using the cell-system model ranged from 25 to 200 m*M*. Thus the "high" ethanol concentrations of in vitro studies may not be comparable to the ethanol levels reached in the brain after chronic ethanol consumption. This topic was elegantly discussed by Deitrich and Harris (1996).

At least eight different isozymes of adenylate cyclase have been identified and characterized recently (Krupinski et al., 1992). These isozymes differ in their primary structures as well as in their response to various modulators of enzymatic activity in vivo and in vitro. More recently, Yoshimura and Tabakoff (1995) studied the effects of acute ethanol exposure on the six adenylate cyclase isozymes that are transiently expressed in human embryonic kidney (HEK 293) cells. They found that prostaglandin E_1 (PGE_1)-stimulated Type I and Type II adenylate cyclase isozymes are insensitive to ethanol. The other isozymes of adenylate cyclase are sensitive to ethanol, and PGE_1-stimulated cAMP formation is increased in HEK 293 cells by acute ethanol exposure. The most important observation of this study was that Type VII adenylate cyclase is the most sensitive to ethanol exposure (50–200 mM ethanol concentrations). These studies also suggest that the differential responses of the activity of adenylate cyclase to ethanol in different brain regions of the rodent may be caused by the presence of specific types of adenylate cyclase in these regions. Future studies are needed to examine the changes in expression of various isozymes of adenylate cyclase in different brain structures of rats and mice during ethanol exposure.

As described above, in the rodent brain the various neurotransmitter receptor and postreceptor steps (G proteins and adenylate cyclase) of the cAMP signal-transduction pathway are altered in the rat brain by chronic ethanol exposure. Furthermore, cAMP-dependent protein phosphorylation via PKA was found to be reduced in striatal membranes of chronic ethanol-treated rats (Rius et al., 1986). This led us and other investigators to examine the changes in the downstream steps of this pathway in the brain during ethanol dependence. One of the key downstream steps of this pathway is the cAMP-responsive-element binding protein (CREB) gene transcription factor, which regulates the expression of several cAMP-inducible genes in the brain (Comb et al., 1987; Collins et al., 1992). CREB is regulated by the cAMP-signaling cascade through phosphorylation by cAMP-dependent PKA (Bito et al., 1996; Sheng et al., 1991). Various other protein kinases, such as calmodulin-dependent kinases, protein kinase C, and casein kinase, also regulate CREB (Gonzales et al., 1989). The dephosphorylation of CREB is regulated by calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase (Bito et al., 1996). We recently observed that CRE-DNA-binding activity in the cortices of rats is slightly decreased (20%) during protracted ethanol exposure, but significantly decreased (56%) during

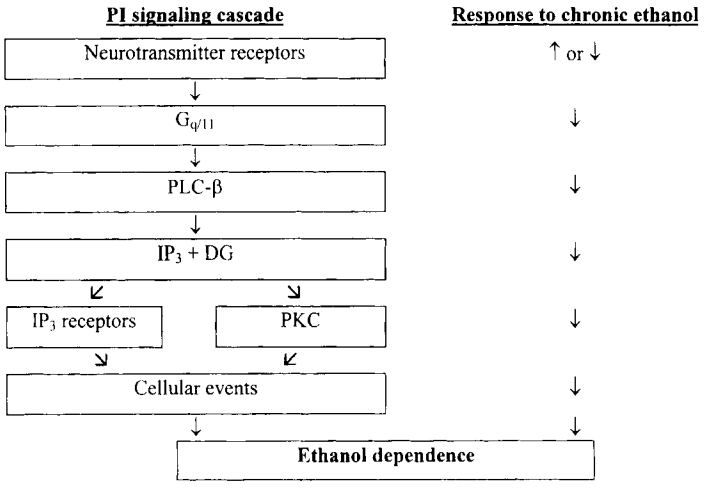

Fig. 2. The PI-signaling cascade in the brain and its responses to chronic ethanol exposure. ↑ = increased; ↓ = decreased. $G_{q/11}$ = GTP binding protein; PLC-β = phospholipase C-β; IP_3 = inositol trisphosphate; DG = diacylglycereol; PKC = protein kinase C.

24 h of ethanol withdrawal after 15 d of ethanol treatment (Pandey et al; 1997). Yang et al., (1997) reported that both phosphorylation of CREB and PKA activity are decreased in the nuclear fraction of the striatum of rats chronically treated with ethanol. They also observed that expression of the proenkephalin gene, which is regulated by CREB, is also decreased in the rat striatum during ethanol exposure. Yang et al. (1996) also reported that acute ethanol exposure (blood ethanol levels, 252 mg%) increases CRE-DNA binding activity, PKA activity, and also phosphorylation of CREB; whereas chronic ethanol exposure (blood ethanol levels, 200 mg%) has no effect on these parameters in the rat cerebellum. Several other genes, such as somatostatin and brain-derived neurotrophic factor (BDNF), have been shown to be regulated by the CREB-gene transcription factor (Gonzales and Montminy, 1989; Condorelli et al., 1994). Interestingly, expression of BDNF and somatostatin genes is also reduced in the rat brain during ethanol exposure (Maclennan et al., 1995; Andrade et al., 1992). These results indicate that CREB and CREB-related genes may be potential molecular targets for the neuroadaptive consequences of chronic ethanol exposure and that these genes may also be vitally involved in mechanisms of ethanol dependence.

The Phosphoinositide-Signaling System: Acute and Chronic Effects of Ethanol

The phosphoinositide-signaling cascade, another important intracellular signaling system, is coupled to several neurotransmitter receptors in the brain (Berridge, 1987; Fisher et al., 1992). The sequence of steps involved in the PI signaling cascade (Fig. 2) is as follows: cell-surface receptor→$G_{q/11}$ protein → the enzyme phospholipase C → second messenger (inositol trisphosphate and diacylglycerol) → protein kinase C → phosphoprotein. Ethanol has been shown to alter each of these steps of the PI-signaling system, either in the cell line or in the brain (Simonsson et al., 1991; Pandey, 1996; Saito et al., 1996a, b).

In an earlier study by Allison and Cicero (1980), acute ethanol administration (peak blood ethanol levels approx 350 mg%) was shown to decrease the level of myoinositol 1-

phosphate (IP_1) in the cerebral cortex of rats. Lin et al. (1993) also demonstrated that acute ethanol administration (peak blood ethanol levels approx 406 mg%) decreases the levels of IP_1 in the mouse cerebrum and cerebellum. Ethanol (500 m*M*) exposure in vitro has been shown to decrease noreprinephrine (NE)-stimulated PI hydrolysis, whereas neither carbachol- nor serotonin-stimulated PI hydrolysis is changed in rat cortical slices (Gonzales and Crews, 1988). Furthermore, ethanol dose dependently (50–500 m*M*) inhibits the muscarinic receptor-stimulated PI hydrolysis in primary cortical cultures of rats (Kovacs et al., 1995). The NE-stimulated PI hydrolysis is not changed; however, the 5-HT-stimulated PI hydrolysis is decreased in cortices of rats treated chronically with ethanol (Pandey and Pandey, 1996). In these studies, blood ethanol levels ranged from 170 to 190 mg%. On the other hand, Hoffman et al. (1986) reported that the EC_{50} for carbachol stimulation of PI hydrolysis is decreased in the cortex, but not in the striatum of mice treated chronically with ethanol. These studies suggest that ethanol has an inhibitory effect on the PI-signaling system in the rodent brain, whereas the site and the nature of ethanol's action on this signaling cascade are still unclear. It is possible that the inhibitory action of ethanol on the PI-signal-transduction system could be related to the inhibition of the enzyme phospholipase C (PLC). We studied PLC activity during chronic ethanol treatment and found decreased PLC activity in the rat cortex (Pandey, 1996). Katsura et al. (1994) also found that chronic ethanol treatment significantly decreased PLC activity in NG108-15 cells and in the cortices of mice. It has been shown that ethanol exposure (25–200 m*M* for 2 d) also inhibits bradykinin receptor-stimulated PI hydrolysis in cultures of NG108-15 cells (Rodriguez et al., 1992). These studies provide some evidence that PLC may be one of the important intracellular sites for the action of ethanol in the brain.

PLC has been categorized into three main families: PLC-β, -γ, -δ (Cockcroft and Thomas, 1992; Rhee and Choi, 1992) Several studies have shown that neurotransmitter receptors are coupled to the PI-signaling system via the $G_{q/11}$ family of G proteins (Rhee and Choi, 1992). It has been demonstrated that PLC-β is regulated by $G_{q/11}$ protein-coupled receptors, whereas PLC-γ is regulated by the occupancy of the tyrosine-kinase receptor; but the mechanism by which the PLC-δ isozyme is regulated is unknown at present (Cockroff and Thomas, 1992; Rhee and Choi, 1992). In one recent study, we examined the changes in protein expression of PLC-β_1, -γ_1 and -δ_1 in the cortices of rats treated acutely (blood ethanol levels approx 90 mg%) or chronically (blood ethanol levels approx 198 mg%) with ethanol. It was found that acute ethanol treatment has no effect on the immunolabeling of PLC-β_1, -γ_1, or -δ_1 isozymes in the rat cortex. On the other hand, immunolabeling of PLC-β_1 but not of -γ_1, or -δ_1 isozymes is significantly decreased in the rat cortex during protracted ethanol exposure (Pandey, 1996). These studies suggest that the decrease in PLC activity during protracted ethanol exposure may be caused by decrease in protein expression of the PLC-β_1 isozyme in the rat cortex. Since PLC-β_1 is regulated by $G_{q/11}$ protein-coupled receptors, we also investigated the changes in expression of the α subunit of $G_{q/11}$ protein in the rat cerebral cortex during ethanol exposure (Pandey, 1996) and found that the expression of the $G_{q/11}$ α protein is decreased in the rat cortex during protracted exposure to ethanol. Interestingly, Zhang et al. (1997) reported that chronic ethanol exposure (peak blood ethanol levels approx 250 mg%) resulted in a significant decrease in inositol monophosphate (IP_1) formation in the cortex and the hippocampus but not in the cerebellum. These results point out that both expression and function of phospholipase C in the rat brain are decreased by chronic ethanol exposure. Simonsson et al. (1991) observed that G-protein-stimulated PI hydrolysis is inhibited by chronic ethanol exposure (100 m*M* for 7 days) in NG108 × 15 cells. Williams and Kelly (1993) measured the immunolabeling of $G_{q/11}$ α protein in NG108 × 15 cells exposed to various concentrations (10–200 m*M*) of ethanol for

48 h and found that only high concentrations of ethanol (100 and 200 m*M*) reduce the levels of $G_{q/11}$ α protein in NG108 × 15 cells. Taken together, all these studies are indicative of the possible involvement of $G_{q/11}$ protein-coupled PLC-β_1 in the neuroadaptational mechanisms to chronic ethanol exposure.

The effects of chronic ethanol exposure on the PI-signaling system in primary cultures of astrocytes has also been studied. It has been reported that chronic ethanol (100 or 200 m*M*) exposure results in an increase in NE- and a decrease in glutamate-stimulated inositol-phosphate formation (Ritchie et al., 1988; Smith, 1994). On the other hand, chronic ethanol exposure has no effect on guanine 5′-(γ-thiotrisphosphate)-stimulated PI hydrolysis in primary cultures of astrocytes (Smith, 1994). The possibility that changes in agonist-stimulated PI hydrolysis during ethanol exposure may be caused by alterations in the expression of PLC isozymes (β_1, γ_1, and δ_1) and/or the expression of the α subunit of $G_{q/11}$ protein was also investigated in primary cultures of astrocytes. It was found that chronic ethanol exposure (100 or 200 m*M* for 4 d) resulted in a significant increase in protein expression of PLC-δ_1, whereas under similar conditions, protein expression of PLC-β_1 (42 kDa) and PLC-γ_1, and of the α subunit of $G_{q/11}$ protein were not significantly altered in astrocytes (Pandey et al., 1996).

These results indicate the molecular mechanisms by which chronic ethanol exposure modulates the PI-signaling system in astrocytes. That a significant increase in protein levels of the PLC-δ_1 isozyme is induced by chronic ethanol exposure suggests that this isozyme may play an important role in the regulation of the PI-signal transduction pathway in astrocytes.

The receptor-coupled hydrolysis of membranal phosphoinositides, particularly phosphatidyl inositol 4,5-bisphosphate (PIP_2), yields two intracellular second messengers, diacylglycerol (DG) and inositol trisphosphate (IP_3). DG activates PKC, and IP_3 mobilizes Ca^{2+} after binding with cytoplasmic IP_3 receptors (Berridge, 1987; 1993; Fisher et al., 1992). These processes appear to be an important part of the signal-transduction mechanism for controlling the various cellular events in the brain (Fig. 2). PKC is a key regulatory enzyme in the brain that modulates both pre- and postsynaptic neuronal functions such as synthesis and release of neurotransmitters, and regulation of receptors and ion channels, neuronal excitability, and gene expression (Nishizuka, 1992). Molecular cloning and biochemical studies have revealed that PKC consists of a family of at least 11 isozymes with different distributions and sensitivities to calcium and to phorbol esters. PKC-α, -βI, -βII, and -γ are conventional isozymes, whereas PKC-δ, -ε, -η, -θ, -μ are novel PKCs. In addition, two atypical isozymes, PKC-ζ and λ, were recently characterized. The conventional isozymes are calcium- and phospholipid-dependent, whereas the novel isozymes do not require calcium for activation. Atypical isozymes are both calcium- and phorbol-ester independent but can be activated by phosphatidylserine (Nishizuka, 1992; Ohno et al., 1991).

In general, acute ethanol exposure (25–200 m*M*) has been shown to stimulate PKC in various cell systems (DePetrillo and Swift, 1992; Skwish and Shain, 1990; DePetrillo and Liou, 1993). The acute physiological effects of ethanol are reversible. After long-term ethanol intake, however, adaptive changes take place in the brain that may alter the acute effects of ethanol on PKC. Both preclinical and clinical findings suggest that adaptive mechanisms during chronic ethanol consumption in vivo are associated with decreased PKC. Clinical studies suggest that PKC activity is lower in lymphocytes of alcoholics compared with normal control subjects (DePetrillo, 1994), but after 5 d of ethanol withdrawal, PKC activity in lymphocytes is similar to that of normal control subjects (DePetrillo, 1994). Preclinical studies suggest that in vivo chronic ethanol exposure (25 d) decreases [^{3}H]phorbol-12, 13-dibutyrate (PDBu) binding to PKC and also decreases PKC activity in the cortex and the hippocampus of the rat brain (Battaini et al.,

1989). Kruger et al. (1993) reported that PKC activity associated with membranes but not with cytosol is decreased in the forebrain of ethanol-treated rats; however, these authors reported an increase in PDBu binding in the CA1 region of the hippocampus but not in other brain regions. The difference in the findings regarding PDBu binding to PKC in the hippocampus and the cortex between their study (Kruger et al., 1993) and other studies (Battaini et al., 1989; Pandey et al., 1993) may be related to the use of different strains of rats.

We studied the effects of chronic ethanol exposure (blood ethanol levels approx 152 mg%) on PKC by [^{3}H]PDBu binding in the rat cortex and observed that chronic ethanol consumption (60 d) leads to the downregulation of PKC associated with membranes but not with cytosol (Pandey et al., 1993). It is not currently known, however, if the decrease in phorbol-ester binding to membranal PKC after chronic alcohol consumption is caused by a decrease in expression of the various PKC isozymes in the brain. Our data on the Western blotting of PKC isozymes indicate that chronic ethanol treatment (15 d) results in decreased protein expression (immunolabeling) of membranal but not cytosolic PKC-α, -β, and -γ isozymes in the rat cortex (Pandey and Alling, 1996). Thus, the decrease in [^{3}H]PDBu binding to membranal PKC during chronic ethanol consumption may be because of a decrease in protein expression of PKC -α, -β, and -γ in the rat cortex. These results suggest a disruption in expression and function of PKC in the brain of ethanol-treated rats.

Messing et al. (1991) studied the effects of chronic ethanol exposure on PKC isozymes in cultured neural (PC-12) cells. They observed that treatment with 100 m*M* ethanol for 6 d increased protein expression (immunolabeling) of PKC-δ and -ε without significantly altering protein expression of PKC-α, -β, or ζ. In addition, they noted that chronic ethanol exposure also increased phorbol-ester binding to PKC and PKC activity in the total fraction of PC-12 cells. In their study, membranal and cytosolic PKC were not isolated; therefore, it is not clear whether membranal or cytosolic (or both) PKC is responsible for the overall increase in PKC in these cells during chronic ethanol exposure. Interestingly, PKC-γ, which is present only in the brain, is absent in PC-12 cells. It is thus possible that the regulation of PKC isozymes in PC-12 cells may not be the same as in the brain in vivo during chronic ethanol consumption. Recently Gordon et al., (1997) reported that ethanol exposure (200 m*M* for 2 d) causes translocation of PKC-δ from Golgi to the perinucleus and of PKC-ε from the perinucleus to the cytoplasm in NG 108–15 cells. All these studies suggest that PKC may be involved in the cellular adaptation to chronic ethanol exposure in PC-12 and NG 108–15 cells and in the brain. Further studies, using immunostaining techniques, are needed to elucidate the localization of the changes in PKC isozymes in various brain structures during acute and chronic ethanol exposure.

As was discussed at the beginning of this section, IP_3 is a major second messenger in the PI signaling cascade and is generated by PIP_2 hydrolysis, which is catalyzed by PLC (Berridge, 1987; Fisher et al., 1992). The binding of IP_3 to a specific IP_3 receptor on the endoplasmic reticulum causes the release of Ca^{2+} from the endoplasmic reticulum (Berridge, 1987; Fisher et al., 1992). Several reports indicate that protracted ethanol exposure can modulate the IP_3 receptor in various rodent brain structures (Saito et al., 1996a; Smith, 1987). Smith (1987) has reported that protracted ethanol exposure causes a decrease in IP_3-receptor binding sites in the cerebellum of mice. This result was recently confirmed by Saito et al. (1996a) in the rat cerebellum. These investigators observed that 24 h of ethanol withdrawal after protracted ethanol exposure significantly decreased IP_3-receptor binding sites without modulating IP_3 mRNA levels in the rat cerebellum. These authors also found that IP_3-receptor binding is decreased in platelets of alcoholic subjects compared with nonalcoholic subjects (Saito et al., 1996b).

Thus, various components of the PI-signaling cascade in the brain are modulated by protracted ethanol exposure. It is reasonable to

speculate that the changes in PLC, PKC, and in IP_3 receptors, which represent the major steps of the PI-signaling transduction pathway (Fig. 2) in the brain, may be involved in the molecular mechanisms associated with the development of ethanol tolerance and/or dependence.

Studies conducted in animal models regarding the changes in cAMP- and PI-signaling pathways during ethanol treatment suggest that these changes may be involved in alcohol tolerance and/or dependence, but do not provide direct evidence regarding the causal relationship between the changes in the signaling cascade and the behavioral effects of ethanol intake and/or withdrawal. Recently, it has been demonstrated that brain adenosinergic modulation of ethanol-induced motor incoordination may be functionally correlated with the changes in the cAMP-signaling cascade at the level of the G_i protein (Saeed Dar, 1997). Future studies are needed to establish a clear role for the neuronal cAMP- and PI-signaling cascades in the behavioral consequences of alcohol tolerance and/or withdrawal.

Modulation of Neurotransmitter Receptors by Acute Ethanol via Protein Kinase C

Ethanol has been shown to interact with a wide variety of transmembranal voltage-gated ion channels and neurotransmitter receptor-regulated channels (Tabakoff and Hoffman, 1996; Allan and Harris, 1987; Grant and Lovinger, 1995). More importantly, recent studies suggest that γ-aminobutyrate type A ($GABA_A$) and N-methyl-D-aspartate (NMDA)-ionotropic receptors are sensitive to ethanol exposure (Allan and Harris, 1987; Grant and Lovinger, 1995). Molecular and biochemical studies have revealed a complex structure of $GABA_A$ receptors, which are a chloride-channel complex consisting of heteromeric multisubunit families (Macdonald and Olsen, 1994). Numerous studies indicate that changes in the subunit composition of $GABA_A$ receptors may be associated with alcohol tolerance and/or dependence (Devaud et al., 1997; Morrow, 1995). The role of the phosphorylation of $GABA_A$ receptors by PKC has been the subject of recent investigations. In this regard, the γ_2 subunit of the $GABA_A$ receptor has been shown to be critical in determining ethanol sensitivity to this receptor. The γ_2 subunit of the $GABA_A$ receptor exists in two forms, γ_{2S} and γ_{2L} subunits. The γ_{2L} but not the γ_{2S} subunit of the $GABA_A$ receptor contains the consensus sequence for phosphorylation by PKC (Glencourse et al., 1992; Whiting et al., 1990). It has been demonstrated that modulation of $GABA_A$ receptors by ethanol may depend on the presence of at least the phosphorylated γ_{2L} subunit in the receptor (Allan and Harris, 1987; Mihic and Harris, 1995; Macdonald, 1995). The $GABA_A$ receptors was shown to be sensitive to even low concentrations of ethanol (5–50 m*M*), and this action of ethanol may depend on the phosphorylation of the receptor by PKC (Pandey and Alling, 1996; Allan and Harris, 1987; Mihic and Harris, 1995). Thus, PKC-mediated phosphorylation appears to be important for the enhancement of $GABA_A$ responses (Fig. 3).

To confirm the role of PKC in the modulation of the $GABA_A$ receptor by ethanol, Harris et al. (1995) used a line of null mutant mice lacking the PKC-γ isozyme in the brain. They observed that these mutant mice display reduced sensitivity to ethanol but normal responses to flunitrazepam and pentobarbital. They also studied the function of the $GABA_A$ receptor in brain membranes isolated from mutant mice and observed that mutation abolished the action of ethanol on the $GABA_A$ receptor but did not affect the actions of flunitrazepam and pentobarbital. These results, as suggested by Harris et al. (1995), clearly demonstrate the role of PKC in the mediation of ethanol's action on the $GABA_A$ receptor.

NMDA receptors are another form of ionotropic receptors and contain integral specific cationic channels (Grant and Lovinger, 1995; Monyer et al., 1994). These receptors have been shown to be sensitive to ethanol concentrations as low as 5 m*M* and also inhibit the long-term

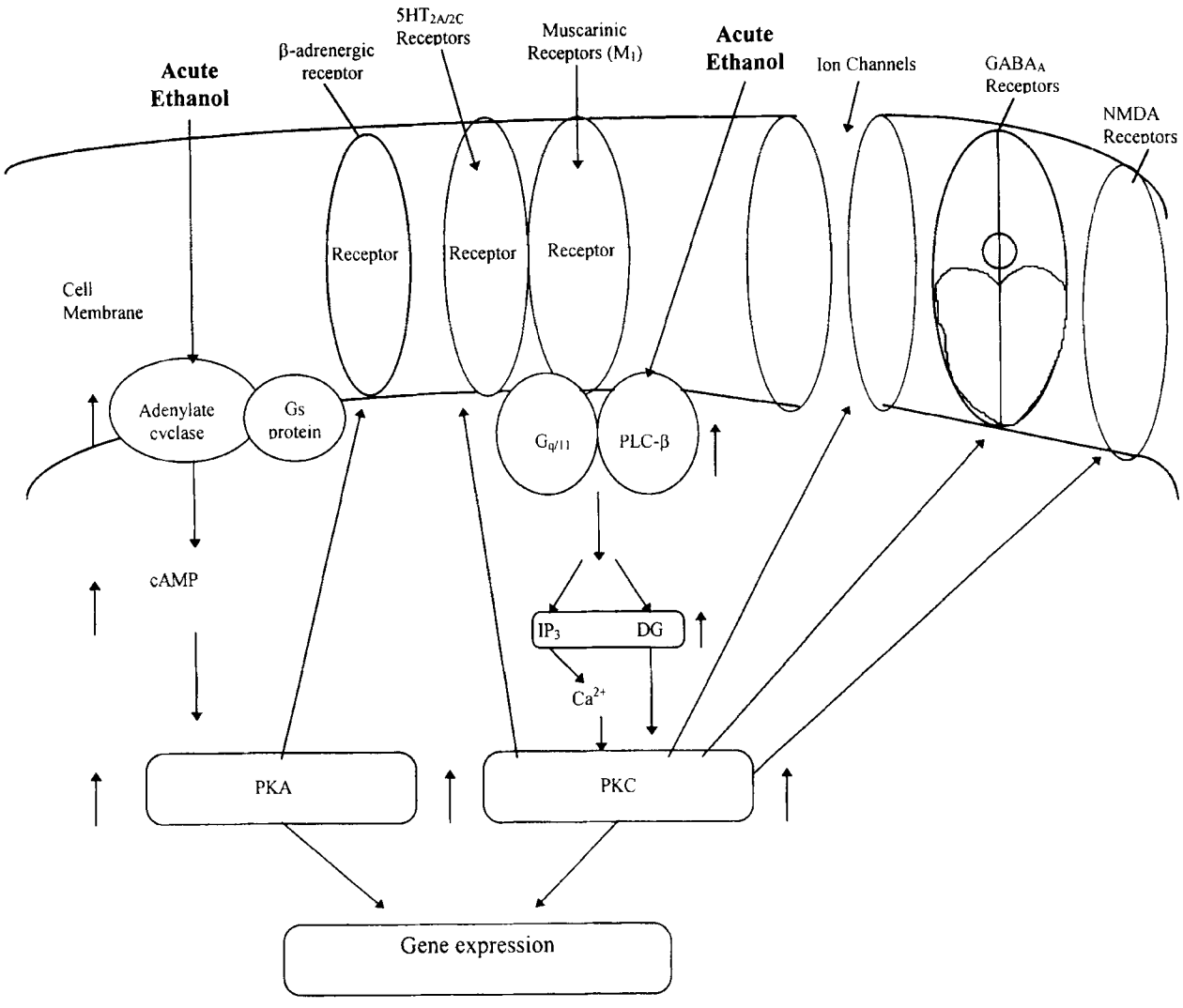

Fig. 3. Modulation of neurotransmitter receptors by acute ethanol exposure via activation of protein kinases A and C. This hypothetical representation indicates that acute ethanol may modulate the function of ionotropic ($GABA_A$ and NMDA) and of metabotropic receptors (5-$HT_{2A/2C}$, M_1 and β-adrenergic receptors) through phosphorylation via activation of PKA or PKC. cAMP = cyclic adenosine 3′, 5′-monophosphate; PKA = protein kinase A; $GABA_A$ = γ-aminobutyrate type A receptor; NMDA = N-methyl-D-aspartate; PLC-β = phospholipase C-β; IP_3 = inositol trisphosphate; DG = diacylglycerol; PKC = protein kinase C.

potentiation in hippocampus (Blizter et al., 1990). Recent evidence suggests that ethanol (50–250 m*M*)-induced inhibition of NMDA responses may be mediated by a mechanism involving PKC (Snell et al., 1994). It is interesting to note that the ethanol sensitivity of NMDA receptors is dependent on PKC in cerebellar granule cells, whereas in cortical cells ethanol sensitivity to NMDA receptors is independent of PKC (Tabakoff, 1995). Available evidence indicates that an increased number of NMDA receptors as measured by [^{3}H] glutamate or [^{3}H] MK 801 binding are associated with development of ethanol-withdrawal seizures after protracted ethanol exposure (Grant et al., 1990; Gulya et al., 1991). Thus, NMDA receptors may modulate the action of acute or chronic ethanol exposure in the brain.

It is evident from some recent investigations that the action of ethanol on some metabotropic neurotransmitter receptors is also mediated by activation of PKC. It has been shown that acute ethanol exposure (150 m*M*) inhibits the function of serotonin$_{2C}$ (5-HT$_{2C}$) and muscarinic m_1 receptors via the activation of PKC (Sann et al., 1994). More recently, it has been shown that the function of 5-HT$_{2A}$ receptors was also inhibited by ethanol (200 m*M*) via activation of PKC (Minami et al., 1997). These studies were carried out in Xenopus oocytes expressing these neurotransmitter receptors. Larsson et al. (1995) reported the effect of ethanol on muscarinic-receptor-stimulated formation of IP_3, which has been studied in human neuroblastoma SH-SY-5Y cells. They observed that the initial peak of IP_3 formation was concentration dependently decreased by acute ethanol exposure, and that this effect was caused by the activation of PKC by ethanol. These studies provide some indication that the initial action of ethanol on neurotransmitter receptors may be mediated by activation of PKC. Thus, ethanol also utilizes the neuronal-signaling cascade in the modulation of the function of ionotropic as well as of metabotropic neurotransmitter receptors.

Summary and Conclusions

The data reviewed here provide convincing evidence that both acute and chronic ethanol exposure modulate the various steps of the PI- and the cAMP-signaling cascades in a variety of cell systems. The acute effects of ethanol on these signaling cascades may mediate the adaptive changes in cAMP- and PI-signaling cascades that underlie the development of ethanol dependence. It is possible that acute ethanol may effect the function of various neurotransmitters in the brain directly or indirectly, via the phosphorylation of receptors by activating the protein kinases (A and C) (Fig. 3). Thus, the acute effect of ethanol is physiologically relevant and ultimately culminates in the development of ethanol tolerance and/or dependence. The growing body of evidence provided here clearly indicates that decreased function of the cAMP- and the PI-signaling systems may be involved in the neuroadaptational mechanisms to chronic ethanol exposure (Figs. 1 and 2). The final molecular events that could be modulated by changes in these signaling cascades in the brain are the function of gene-transcription factors and gene expression, which could be altered during ethanol dependence. One such example could be CREB and CREB-related genes in the brain. In general, the behavioral impact of ethanol's actions includes the development of ethanol withdrawal symptoms such as anxiety, seizures, and tremors. It is unknown if the changes in neuronal-signaling cascades are associated with the development of these ethanol withdrawal symptoms. Future studies should be directed toward elucidating the association of the behavioral consequences of ethanol's effects with the changes in neuronal-signaling cascades in the brain. Study of the cellular and/or molecular changes in the cAMP- and the PI-signaling cascades in the brain during ethanol withdrawal after chronic ethanol intake is important to provide a basis to develop better drugs to prevent or treat the ethanol withdrawal symptoms.

Acknowledgment

S.C. Pandey is supported by funds from the National Institute on Alcohol Abuse and Alcoholism (Grant AA 10005).

References

Allan A. M. and Harris R. A. (1987) Acute and chronic ethanol treatments alter GABA receptor-operated chloride channels. *Pharmacol. Biochem. Behav.* **27**, 665–670.

Alling C., Gutavsson L., Larsson C., Lundquist C., Rodriguez D., and Simonsson P. (1994) Adaptation of signal transduction in brain, in *Toward a Molecular Basis of Alcohol Use and Abuse.* (Jansson B., Jornvall H., Rydberg U., Terenius L. and Vallee B. L. (eds.) Birkhauser Verlag, Basal, Switzerland, pp. 19–28.

Allison J. H. and Cicero T. J. (1980) Alcohol acutely depresses myo-inositol 1-phosphate levels in the male rat cerebral cortex. *J. Pharmacol. Exp. Ther.* **213**, 24–27.

Andrade J. P., Fernando P. M., Madeira M. D., Paula-Barbosa M. M., Cadete-Leite A., and Zimmer J. (1992) Effects of chronic alcohol consumption and withdrawal on the somatostatin-immunoreactive neurons of the rat hippocampal dentate hilus. *Hippocampus* **2**, 65–71.

Battaini F., Vesco, R. D., Govoni S., and Trabucchi M. (1989) Chronic alcohol intake modifies phorbol ester binding in selected rat brain areas. *Alcohol* **6**, 169–172.

Berridge M. J. (1987) Inositol trisphosphate and diacyl glycerol: two interacting second messengers. *Ann. Rev. Biochem.* **56**, 159–193.

Berridge M. J. (1993) Inositol trisphosphate and calcium signaling. *Nature* **361**, 315–325.

Bito H., Deisseroth K., and Tsien R. W. (1996) CREB phosphorylation and dephosphorylation: a Ca2+- and stimulus duration-dependent switch for hippocampal gene expression. *Cell* **87**, 1203–1214.

Blizter R. D., Gil, O., and Landau E. M. (1990) Long-term potentiation in rat hippocampus is inhibited by low concentrations of ethanol. *Brain Res.* **537**, 203–208.

Bode D. C. and Molinoft P. B. (1988). Effects of ethanol in vitro on the beta-adrenergic receptor-coupled adenylate cyclase system. *J. Pharmacol. Exp. Ther.* **246**, 1040–1047.

Cockcroft S. and Thomas M. H. (1992) Inositol-lipid-specific phospholipase C isoenzymes and their differential regulation by receptors. *Biochem. J.* **288**, 1–14.

Coe I. R., Dohrman D. P., Constantinescu A., Diamond I., and Gordon A. S. (1996) Activation of cyclic AMP-dependent protein kinase reverses tolerance of a nucleotide transporter to ethanol. *J. Pharmacol. Exp. Ther.* **276**, 365–369.

Collins S., Caron M. G., and Lefkowitz R. J. (1992) From ligand binding to gene expression: new insights into the regulation of G protein-coupled receptors. *TIPS* **1**, 37–39.

Comb M., Hyman S. E., and Goodman H. M. (1987) Mechanism of trans-synaptic regulation of the gene expression. *TINS* **10**, 473–478.

Condorelli D. F., Dell' Albani P., Muelo G., Timmusk T., and Belluardo N. (1994) Expression of neurotrophins and their receptors in primary astroglial cultures. Induction by cyclic AMP-elevating agents. *J. Neurochem.* **63**, 509–516.

Deitrich R. A. and Harris R. A. (1996) How much alcohol should I use in my experiments? *Alcohol: Clin. Exp. Res.* **20**, 1–2.

DePetrillo P. B. (1994) Effects of chronic alcohol use and age on human lymphocyte protein kinase C activity. *Pharmacol. Biochem. Behav.* **48**, 999–1004.

DePetrillo P. B. and Liou C. S. (1993) Ethanol exposure increases total protein kinase C activity in human lymphocytes. *Alcohol. Clin. Exp. Res.* **17**, 351–354.

DePetrillo P. B. and Swift R. M. (1992) Ethanol exposure results in a transient decrease in human platelet cAMP levels: evidence for a protein kinase C mediated process. *Alcohol. Clin. Exp. Res.* **16**, 290–294.

Devaud L. L., Fristschy J-M., Sieghart W., and Morrow A. L. (1997) Bidirectional alterations of $GABA_A$ receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. *J. Neurochem.* **69**, 126–130.

Diamond I., Wrubel B., Estrin W., and Gordon A. (1987) Basal and adenosine receptor-stimulated levels of cAMP are reduced in lymphocytes from alcoholic patients. *Proc. Natl. Acad. Sci. USA* **84**, 1413–1416.

Fisher S. K., Heacock A. M., and Agranoff B. W. (1992) Inositol lipids and signal transduction in the nervous system: an update. *J. Neurochem.* **58**, 18–38.

Gilman A. G. (1989) G proteins and regulation of adenylyl cyclase. *JAMA* **6**, 1819–1825.

Glencourse T. A., Bateson A. N., and Darlison M. G. (1992) Differential localization of two alternatively spliced $GABA_A$ receptor γ_2-subunit mRNA's in chick brain. *Eur. J. Neurosci* **4**, 271–277.

Goldstein D. B. (1976) Pharmacological aspects of physical dependence on ethanol. *Life Sci.* **18**, 553–561.

Gonzales G. A. and Montminy M. R. (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serin. *Cell* **59**, 675–680.

Gonzales G. A., Yamamoto K. K., Fischer W. H., Karr D., Menzel P., Biggs W., Vale W. W., and Montminy M. R. (1989) A cluster of phosphorylation sites on the cyclic AMP-regulated nuclear factor CREB predicted by its sequences. *Nature* **337**, 749–752.

Gonzales R. A. and Crews F. T. (1988) Effects of ethanol in vivo and in vitro on stimulated phosphoinositide hydrolysis in rat cortex and cerebellum. *Alcohol. Clin. Exp. Res.* **12**, S94–S98.

Gordon A. S., Collier K. and Diamond I. (1986) Ethanol regulation of adenosine receptor-stimulated cAMP levels in a clonal neuronal cell line: an in vitro model of cellular tolerance to ethanol. *Proc. Natl. Acad. Sci. USA* **83**, 2105–2108.

Gordon A. S., Yao L., Wu Z., Coe I. R., and Diamond I. (1997) Ethanol alters the subcellular localization of δ-and ε-protein kinase C in NG 108–15 Cells. *Mol. Pharmacol.* **52**, 554–559.

Grant K. A. and Lovinger D. M. (1995) Cellular and behavioral neurobiology of alcohol. *Clin. Neurosci.* **3**, 155–164.

Grant K. A., Valverius P., Hudspith M., and Tabakoff B. (1990) Ethanol withdrawal seizures and NMDA receptor complex. *Eur. J. Pharmacol.* **176**, 289–296.

Gulya K., Grant K. A., Valverius P., Hoffman P. L., and Tabakoff B. (1991) Brain regional specificity and time course of changes in the NMDA receptor-ionophore complex during ethanol withdrawal. *Brain Res.* **547**, 129–134.

Harris R. A. and Buck K. J. (1990) The processes of alcohol tolerance and dependence. *Alcohol World Health Res.* **14**, 105–110.

Harris R. A., McQuilkin S. J., Paylor R., Abeliovich A., Tonegawa S., and Wehner J. M. (1995) Mutant mice lacking the γ-isoform of protein kinase C show decreased behavioral actions of ethanol and altered functions of γ-aminobutyrate type A receptors. *Proc. Natl. Acad. Sci. USA* **92**, 3658–3662.

Hoffman P. L. and Tabakoff B. (1990) Ethanol and guanine nucleotide binding proteins: a selective interaction. *Fed. Am. Soc. Exp. Biol. J.* **4**, 2612–2622.

Hoffman P. L., Moses F., Luthin G. R., and Tabakoff B. (1986) Acute and chronic effects of ethanol on receptor-mediated phosphatidylinositol 4,5-bisphosphate breakdown in mouse brain. *Mol. Pharmacol.* **30**, 13–18.

Katsura M., Ohkuma S., Chen D.-Z., and Kuriyama K. (1994) Ethanol-induced alteration in activities of cerebral phosphatidylinositol 4,5-bisphosphate specific and cytosolic phospholipase C in the brain: analysis using NG 108–15 cells and brains from ethanol-inhaled mice. *Neurochem. Int.* **24**, 541–547.

Kovacs K. A., Kavanagh T. J., and Costa L. G. (1995) Ethanol inhibits muscarinic receptor-stimulated phosphoinositide metabolism and calcium mobilization in rat primary cortical cultures. *Neurochem. Res.* **20**, 939–949.

Kruger H., Wilce P. A., and Shanley B. C. (1993) Ethanol and protein kinase C in rat brain. *Neurochem. Int.* **22**, 575–581.

Krupinski J., Lehman T. C., Frankenfield C. D., Zwaagstra J. C., and Watson P. A. (1992) Molecular diversity in the adenylyl cyclase family: evidence for eight forms of the enzyme and cloning of type VI. *J. Biol. Chem.* **267**, 24858–24862.

Larsson C., Simonsson P., Hoek J. B., and Alling C. (1995) Ethanol inhibits the peak of muscarinic receptor-stimulated formation of inositol 1,4,5-trisphosphate in neuroblastoma SH-SY-5Y cells. *Biochem. Pharmacol.* **50**, 647–654.

Lichtenberg-Kraag B., May T., Schmidt L. G., and Rommelspacher H. (1995) Changes of G protein levels in platelet membranes from alcoholics during short-term and long-term abstinence. *Alcohol Alcohol* **30**, 455–464.

Lin T-A., Navidi M., James W., Lin T-N., and Sun G. Y. (1993) Effects of acute ethanol administration on polyphosphoinositide turnover and levels of inositol 1,4,5-trisphosphate in mouse cerebrum and cerebellum. *Alcohol. Clin. Exp. Res.* **17**, 401–405.

Macdonald R. L. (1995) Ethanol, γ-aminobutyrate type A receptors and protein kinase C phosphorylation. *Proc. Natl. Acad. Sci. USA* **92**, 3633–3635.

Macdonald R. L. and Olsen R. W. (1994) $GABA_A$ receptor channels. *Ann. Rev. Neurosci.* **17**, 569–602.

MacLennan A. J., Lee N., and Walker D. W. (1995) Chronic ethanol administration decreases brain-derived neurotrophic factor gene expression in the rat hippocampus. *Neurosci. Letter.* **197**, 105–108.

McKenzie F. R. and Milligan G. (1990) Prostaglandin E_1-mediated, cyclic AMP-independent, downregulation of $G_s\alpha$ in neuroblastoma x glioma hybrid cells. *J. Biol. Chem.* **265**, 17084–17093.

Messing R. O., Peterson P. J., and Henrich C. J. (1991) Chronic ethanol exposure increases levels of protein kinase C δ, ε and protein kinase C-mediated phosphorylation in cultured neural cells. *J. Biol. Chem.* **266**, 23,428–23,432.

Mihic S. J. and Harris R. A. (1995) Alcohol actions at the $GABA_A$ receptor/chloride channel complex, in *Pharmacological Effects of Ethanol on the Nervous System*. Deitrich R. A. ed. CRC Press, Boca Raton, FL pp. 51–72.

Minami K., Minami M., and Harris R. A. (1997) Inhibition of 5-hydroxytryptamine type 2A receptor-induced currents by n-alcohols and anesthetics. *J. Pharmacol. Exp. Ther.* **281**, 1136–1143.

Monyer H., Burnashev N., Laurie D. J., Sakmann B., Seeburg P. H. (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**, 529–540.

Morrow, A. L. (1995) Regulation of $GABA_A$ receptor function and gene expression in the central nervous system. *Int. Rev. Neurobiol.* **38**, 1–41.

Nagy L. E., Diamond I., and Gordon A (1988) Cultured lymphocytes from alcoholic subjects have altered cAMP signal transduction. *Proc. Natl. Acad. Sci. USA* **85**, 6973–6976.

Neer E. J. (1995) Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* **80**, 249–257.

Nishizuka Y. (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258**, 607–614.

Ohno S., Akita Y., Hata A., Osada S-C., Kubo K., Konno Y., Akimoto K., Mizuno K., Saito T., Kuroki T., and Suzuki K. (1991) Structural and functional diversities of a family of signal transducing protein kinases, protein kinase C family; two distinct classes of PKC, conventional PKC and novel nPKC. *Adv. Enzyme Regulation* **31**, 287–303.

Pandey S. C, Dwivedi Y., Piano M. R., Schwartz D. W., Davis J. M., and Pandey G. N. (1993) Chronic ethanol consumption decreases the phorbol ester binding to membranal but not cytosolic protein kinase C in rat brain. *Alcohol* **10**, 259–262.

Pandey S. C. (1996) Acute and chronic ethanol consumption effects on the immunolabeling of $G_{q/11}$ α subunit protein and phospholipase C isozymes in the rat brain. *J. Neurochem.* **67**, 2355–2361.

Pandey S. C. and Alling C. (1996) Protein kinase C: molecular and cellular targets for the action of ethanol. *Alcohol. Clin. Exp. Res.* **20**, 67A–71A.

Pandey S. C. and Pandey G. N. (1996) Modulation of $serotonin_{2A/2C}$ receptors and these receptor-linked phosphoinositide system by ethanol. *Behav. Brain Res.* **73**, 235–238.

Pandey S. C., Pandey G. N., and Smith T. L. (1996) Chronic ethanol effects on the expression of phospholipase C isozymes and $G_{q/11}$ protein in primary cultures of astrocytes. *Alcohol* **13**, 487–492.

Pandey S. C., Zhang D., and Nayyar D. (1997) Acute and protracted ethanol consumption effects on the CREB and AP-1 gene transcription factors in the rat brain. *Alcohol. Clin. Exp. Res.* **21(3)**, 76A.

Rabin R. A. (1993) Ethanol-induced desensitization of adenylate cyclase: role of the adenosine receptor and GTP-binding proteins. *J. Pharmacol. Exp. Ther.* **264**, 977–983.

Rhee S. G. and Choi K. D. (1992) Regulation of inositol phospholipid-specific phospholipase C isozymes. *J. Biol. Chem.* **267**, 12393–12396.

Ritchie T., Kim H.S., Cole R., DeVellis J., and Noble E. P. (1988) Alcohol-induced alterations in phosphoinositide hydrolysis in astrocytes. *Alcohol* **5**, 183–187.

Rius R. A., Govoni S., Battaini F., and Trabucchi M. (1986) Cyclic AMP-dependent protein phosphorylation is reduced in rat striatum after chronic ethanol treatment. *Brain Res.* **365**, 355–359.

Robin R. A. (1990) Chronic ethanol exposure of PC12 cells alters adenylate cyclase activity and intracellular cyclic AMP content. *J. Pharmacol. Exp. Ther.* **252**, 1021–1027.

Rodriguez F. D., Simonsson P., Gustavsson L., and Alling C. (1992) Mechanisms of adaptation to the effects of ehtanol on activation of phospholipase C in NG 108–15 cells. *Neuropharmacology* **31**, 1157–1164.

Saeed Dar M. (1997) Mouse cerebellar adenosinergic modulation of ethanol-induced motor incoordination: possible involvement of cAMP. *Brain Res.* **749**, 263–274.

Saito H., Nishida A., Shimizu M., Motohashi N., and Yamawaki S. (1996a) Effects of chronic ethanol treatment on inositol 1,4,5-trisphosphate receptors and inositol 1,3,4,5-tetrabisphosphate receptors in the rat brain. *Neuropsychobiology* **33**, 60–65.

Saito H., Nishida A., Shimizu M., Motohashi N., and Yamawaki S. (1996b) Decreased inositol 1,4,5-trisphosphate-specific binding in platelets from alcoholic subjects. *Biol. Psychiatr.* **40**, 886–891.

Saito T., Lee J. M., and Tabakoff B. (1985) Ethanol's effects on cortical adenylate cyclase activity. *J. Neurochem.* **44**, 1037–1044.

Saito T., Lee J. M., Hoffman P. L., and Tabakoff B. (1987) Effects of chronic ethanol treatment on the beta-adrenergic receptor-coupled adenylate cyclase system of mouse cerebral cortex. *J. Neurochem.* **48**, 1817–1822.

Sann E., Dildy-Mayfield J. E., and Harris R. A. (1994) Ethanol inhibits the function of 5-hydroxytryptamine type 1C and muscarinic M_1 G protein-linked receptors in xenopus oocytes expressing brain mRNA: role of protein kinase C. *Mol. Pharmacol.*, **45**, 1004–1012.

Sheng M., Thompson M. A., and Greenberg M. E. (1991) CREB: a Ca2+ - regulated transcription factor phosphorylated by calmodulin-dependent kinases. *Science* **252**, 1427–1430.

Simonsson P., Rodriguez F. D., Loman N., and Alling C. (1991) G proteins coupled to phospholipase C: molecular targets of long-term ethanol exposure. *J. Neurochem.* **56**, 2018–2026.

Skwish S. and Shain W. (1990) Ethanol and diolein stimulated PKC translocation in astroglial cells. *Life Sci.* **47**, 1037–1042.

Smith T. L. (1994) Selective effects of ethanol exposure on metabotropic glutamate receptor and guanine neucleotide stimulated phospholipase C activity in primary cultures of astrocytes. *Alcohol* **11**, 405–409.

Smith T. L. (1987) Chronic ethanol consumption reduces [3H]inositol 1,4,5 trisphosphate specific binding in mouse cerebellar membrane fragments. *Life Sci.* **41**, 2863–2868.

Snell L. D., Tabakoff B., and Hoffman P. L. (1994) Involvement of protein kinase C in ethanol induced inhibition of NMDA receptor function in cerebellar granule cells. *Alcohol. Clin. Exp. Res.* **18**, 81–85.

Sun G. Y. and Sun A. Y. (1985) Ethanol and membrane lipids. *Alcohol. Clin. Exp. Res.* **9**, 164–180.

Tabakoff B. and Rothstein J. D. (1983) Biology of tolerance and dependence in *Medical and Social Aspects of Alcohol Abuse.* (Tabokoff B, Sutker P. B., and Randall C. L., eds.) Plenum, New York, pp. 197–221.

Tabakoff B. (1995) Ethanol's action on the $GABA_A$ receptor: is there a requirement for parsimony? *Alcohol Clin. Exp. Res.* **19**, 1597–1598.

Tabakoff B., Cornell N., and Hoffman, P. L. (1986) Alcohol tolerance. *Ann. Emerg. Med.* **15**, 1005–1012.

Tabakoff B. and Hoffman P. L. (1996) Alcohol addiction: an enigma among us. *Neuron.* **16**, 909–912.

Tabakoff B., Hoffman P. L., Lee J. M., Saito T., Willard B., and DeLeon-Jones F. (1988) Differences in platelet enzyme activity between alcoholics and nonalcoholics. *N. Engl. J. Med.* **318**, 134–139.

Tabakoff B., Whelan J. P., Ovchinnikova L., Nhamburo P., Yoshimura M., and Hoffman P. L. (1995) Quantitative changes in G proteins do not mediate ethanol-induced downregulation of adenylyl cyclase in mouse cerebral cortex. *Alcohol. Clin. Exp. Res.* **19**, 187–194.

Valverius P., Hoffman P. L. and Tabakoff B. (1989a) Hippocampal and cerebellar β-adrenergic receptors and adenylate cyclase are differentially altered by chronic ethanol ingestion. *J. Neurochem.* **52**, 492–497.

Valverius D., Hoffman P. L. and Tabakoff B. (1989b) Brain forskolin binding in mice dependent on and tolerant to ethanol. *Brain Res.* **503**, 38–43.

Waltman C., Levine M. A., McCaul M. E., Svikis D. S., and Wand G. S. (1993) Enhanced expression of the inhibitory protein G_{i2} α and decreased activity of adenylyl cyclase in lymphocytes of abstinent alcoholics. *Alcohol. Clin. Exp. Res.* **17**, 315–320.

Wand G. S. and Levine M. (1991) Hormonal tolerance to ethanol is associated with decreased expression of the GTP-binding protein $G_s\alpha$ and adenylate cyclase activity in LS mice. *Alcohol Clin. Exp. Res.* **15**, 705–710.

Wand G. S., Diehl A. M., Levine M. A., Wolfgang D., and Samy S. (1991) Chronic ethanol treatment increases expression of inhibitory G proteins and adenylate cyclase activity in the CNS of two lines of ethanol-sensitive mice. *J. Biol. Chem.* **268**, 2595–2601.

Wand G. S., Waltman C., Martin C. S., McCaul M. E., Levine M. A., and Wolfgang D. (1994) Differential expression of guanosine triphosphate binding proteins in men at high and low risk for the future development of alcholism. *J. Clin. Invest.* **94**, 1004–1011.

Whiting P., McKernan R. M., and Iverson L. L. (1990) Another mechanism for creating diversity in γ-aminobutyrate A receptors: RNA splicing directs expression of two forms of the γ_2 subunit, one of which contains a protein kinase C phosphorylation site. *Proc. Natl. Acad. Sci. USA* **87**, 9966–9970.

Williams R. J. and Kelly E. (1993) Chronic ethanol reduces immunologically detectable $G_{q\alpha/11\alpha}$ in NG 108–15 cells. *J. Neurochem.* **61**, 1163–1166.

Yang X., Diehl A. M., and Wand G. S. (1996) Ethanol exposure alters the phosphorylation of cyclic AMP responsive element binding activity in rat cerebellum. *J. Pharmacol. Exp. Therap.* **278**, 338–346.

Yang X., Horn K., and Wand G. S. (1997) Chronic ethanol exposure impairs phosphorylation of CREB in rat striatum. *Alcohol. Clin. Exp. Res.* **21(3)**, 40A.

Yoshimura M. and Tabakoff B. (1995) Selective effects of ethanol on the generation of cAMP by particular members of the adenylyl cyclase family. *Alcohol. Clin. Exp. Res.* **19**, 1435–1440.

Zhang J.-P., Xia J.-M., and Sun G. Y. (1997) Chronic ethanol inhibits inositol metabolism in specific brain regions. *Alcohol. Clin. Exp. Res.* **21**, 716–720.