Influence of ethanol on calcium, inositol phospholipids and intracellular signalling mechanisms

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Summary. Studies have implicated Ca^{++} in the actions of ethanol at many biochemical levels. Calcium as a major intracellular messenger in the central nervous system is involved in many processes, including protein phosphorylation enzyme activation and secretion of hormones and neurotransmitters. The control of intracellular calcium, therefore, represents a major step by which neuronal cells regulate their activities. The present review focuses on three primary areas which influence intracellular calcium levels; voltage-dependent Ca^{++} channels, receptor-mediated inositol phospholipid hydrolysis, and Ca^{++}/Mg^{++}-ATPase, the high affinity membrane Ca^{++} pump.
Current research suggests that a subtype of the voltage-dependent Ca^{++} channel, the dihydropyridine-sensitive Ca^{++} channel, is uniquely sensitive to acute and chronic ethanol treatment. Acute exposure inhibits, while chronic ethanol exposure increases $^{45}Ca^{++}$-influx and [^{3}H]dihydropyridine receptor binding sites. In addition, acute and chronic exposure to ethanol inhibits, then increases Ca^{++}/Mg^{++}-ATPase activity in neuronal membranes. Changes in Ca^{++} channel and Ca^{++}/Mg^{++}-ATPase activity following chronic ethanol may occur as an adaptation process to increase Ca^{++} availability for intracellular processes. Since receptor-dependent inositol phospholipid hydrolysis is enhanced after chronic ethanol treatment, subsequent activation of protein kinase-C may also be involved in the adaptation process and may indicate increased coupling for receptor-dependent changes in Ca^{++}/Mg^{++}-ATPase activity.
The increased sensitivity of three Ca^{++}-dependent processes suggest that adaptation to chronic ethanol exposure may involve coupling of one or more of these processes to receptor-mediated events.
Key words. Calcium; inositol phospholipids; Ca^{++} channels; Ca^{++}/Mg^{++}-ATPase; dihydropyridine receptors.

Interactions between membranes and calcium

The effects of ethanol on the central nervous system (CNS) are believed to result from interaction with the neuronal membrane[61]. This action may be directed toward pre- and/or post-synaptic events including synthesis, turnover, release and binding of transmitters to receptor sites which are coupled to effector units in the membrane[62]. Earlier studies suggested that ethanol produces its effects by altering membrane fluidity. Tolerance to this effect occurs when the membrane fluidity and function are restored to the predrug condition. This restoration may result from optimal interactions between cations and the membrane; these interactions may regulate polar-nonpolar phase transitions. Ethanol alters neuronal membrane fluidity which may underlie changes in ion permeability, enzyme activity, and synaptic transmission[7]. The interaction of ethanol with many of these parameters may lead to pharmacological responses, such as hypothermia, sedation, and changes in locomotor activity. The degree to which ethanol causes changes in neuronal membrane fluidity has been correlated with its lipid solubility properties[39]. However, Harris and Bruno have recently reported that the effects of ethanol and other lipid-soluble agents on Ca^{++} uptake into synaptosomes correlated poorly with their membrane disordering effects[23].
Of the many parameters which have been investigated with regard to ethanol and the CNS, changes in Ca^{++} transport continue to receive attention. Ca^{++} content and binding, as well as Ca^{++} transport, have been investigated in both acute and chronic ethanol exposure[13, 22, 41, 49]. It is well known that Ca^{++} in physiological concentrations may stabilize membranes and that Ca^{++}-membrane interactions may be disrupted during ethanol-induced changes in membrane fluidity[64]. These studies suggest that Ca^{++} may play an underlying role in the fluidity changes effected by ethanol, which may be important in interpreting the ethanol effect on the CNS[51].

Ethanol influence on Ca^{++}/Mg^{++}-ATPase

The importance of Ca^{++} in neurotransmission is well documented. Ca^{++} is implicated in both structural and functional activities including membrane stability, enzyme activation, ion conductance, and phosphorylation-dependent transmitter release[50]. Many of these events depend on optimal levels of cytosolic Ca^{++}, which serves as an intracellular signal. Ethanol acts at various levels of synaptic transmission affecting release, turnover, and enzyme coupling. Many of these events require optimal levels of intracellular Ca^{++}. It is important to consider the effect of ethanol on intracellular Ca^{++} levels to understand how altered Ca^{++} signals may affect synaptic transmission[32].
A variety of intracellular Ca^{++} buffers exist to regulate cytosolic Ca^{++}, including its uptake into mitochondria[24] and smooth endoplasmic reticulum[6], and Na^+/Ca^{++} exchange[5]. In addition, an outwardly directed plasma membrane Ca^{++}/Mg^{++}-ATPase[29, 51, 59] exists which is activated by calmodulin and Ca^{++}[51, 59]. While studies have previously focused on ethanol and the Na^+/K^+ pump, recent studies have investigated Ca^{++}-ATPase and various buffer mechanisms. Studies in our laboratory[53] demonstrated that ethanol in vivo produces

an increase in Ca^{++}/Mg^{++}-ATPase and locomotor activity in mice. Logan and Laverty[35] also demonstrated increased Ca^{++}-ATPase activity following low ethanol doses in vivo. Recent studies[18] have correlated loss and recovery of the righting reflex with inhibition and recovery of Ca^{++}/Mg^{++}-ATPase; an effect related to BAC. Using lysed synaptosomal membranes, we demonstrated[18] a correlation between recovery of Ca^{++}/Mg^{++}-ATPase activity and righting reflex, while ATP-dependent Ca^{++} uptake was still inhibited. Chronic treatment has been shown to increase Ca^{++}/Mg^{++}-ATPase in different brain regions, most notably the hypothalamus[47]. This increase was shown to parallel tolerance to thermoregulation changes induced by ethanol. Keane and Leonard[31] also demonstrated regional brain enzyme changes following both acute and chronic ethanol administration. Acute ethanol treatment decreased Ca^{++}/Mg^{++}-ATPase in hippocampus, while chronic treatment increased enzyme activity. No changes were seen in midbrain or amygdala. Adaptive increases in Ca^{++}/Mg^{++}-ATPase may be the result of neuronal responsiveness to the continued presence of ethanol in order to increase Ca^{++} turnover across the plasma membrane and reset the Ca^{++} level for higher neuronal activity.

Ethanol influence and ion channels

Recent studies have suggested that an additional major site for alcohol action is the Ca^{++} channel protein in neuronal membranes. Pharmacologically relevant concentrations of ethanol have been shown to inhibit voltage-dependent Ca^{++}-influx in synaptosomal preparations[10]. Following chronic ethanol administration, a number of laboratories have reported increased Ca^{++} sensitivity and related functional changes in transmitter release consistent with some type of adaptation phenomenon.

Lynch and Littleton[37] first reported an increase in Ca^{++} sensitivity in nerve terminals associated with increased fractional release of neurotransmitters as a result of alcohol tolerance. These investigators suggested that increased sensitivity to Ca^{++} entry may underlie the observed changes in transmitter release. However, in a subsequent study[38] the results suggested that enhanced sensitivity of transmitter release may not be dependent on increased Ca^{++} entry. A similar study was also reported by Leslie et al.[33] in which Ca^{++} entry into synaptosomes following chronic treatment was unaltered.

More recent studies have focused on a subtype of the voltage-sensitive Ca^{++} channel. Greenberg and Cooper[21] reported that ethanol in vitro could reduce the number, but not affinity of dihydropyridine receptor sites. [^{3}H]nitrendipine binding was inhibited in vitro at a $K_i = 460$ mM. While this concentration is far higher than that involved in pharmacological actions of ethanol (25–200 mM), more recent studies have appeared which have studied the effect of ethanol on dihydropyridine-sensitive Ca^{++} channels in greater depth.

Lucchi et al.[36] reported that chronic ethanol treatment increased [^{3}H]nitrendipine binding, while recently Rius et al.[48] reported short-term increases in [^{3}H]nitrendipine receptor number following acute ethanol treatment. Using a clonal cell line of neural crest origin, Greenberg and colleagues have studied acute and chronic exposure to ethanol and dihydropyridine receptor binding and Ca^{++}-influx. Messing et al.[40] have shown that both acute and chronic ethanol exposure decrease, then increase $^{45}Ca^{++}$-influx, and that there are increases in [^{3}H]nitrendipine receptor binding following chronic treatment. Following ethanol removal, $^{45}Ca^{++}$-influx values returned to control levels. These studies suggest that increases in $^{45}Ca^{++}$-influx and [^{3}H]nitrendipine may occur as a result of acute inhibition of binding and influx, and signal adaptation phenomena.

The increased sensitivity of the dihydropyridine-dependent Ca^{++} channel was reported by Dolin et al.[11] using rat brain membranes. Following chronic ethanol treatment in rats, [^{3}H]nimodipine receptor binding in the cortex was increased, together with BAY K8644-dependent inositol lipid hydrolysis. These findings suggest that both receptor binding and functionality for the dihydropyridine-dependent class of Ca^{++} channels are altered following chronic treatment. BAY K8644-dependent Ca^{++} entry would be expected to stimulate phospholipase-C activity, with subsequent hydrolysis of inositol phospholipids. Thus these data suggest increased Ca^{++} entry, similar to that reported for PC-12 cells by Messing et al.[40]. Greenberg et al.[20] have confirmed these studies and demonstrated that Ca^{++} channel antagonists acting on the dihydropyridine-sensitive Ca^{++} channel were effective in blocking Ca^{++} uptake induced by ethanol. Again, these studies suggest that the dihydropyridine-sensitive Ca^{++} channels may undergo adaptation to the continued presence of ethanol. Studies in our laboratory have explored brain regional differences in [^{3}H]nitrendipine receptor binding in chronically ethanol-treated rats[17]. In animals made ethanol tolerant (as evidenced by adaptation to thermoregulation changes), [^{3}H]nitrendipine receptor binding was significantly increased in the hippocampus (33.8%), while decreases were seen in the hypothalamus (15.7%), striatum (23%), and brain stem (21.8%). The regional differences noted here may suggest that under chronic ethanol influence, the adaptative changes that are reflected by changes in receptor number in certain regions, may result in certain brain functions being modified in the continued presence of ethanol, while others are not.

Ethanol and membrane lipids

Ethanol has been known to cause disorder in the cell membrane by interacting with lipid bilayer[7, 25, 34, 56] and thus may influence various membrane functions including receptor binding and transmembrane signalling. The purpose of this section is to describe how ethanol affects the lipid-dependent second messenger system in neuronal

cells. Many cell types possess two types of membrane receptors; the binding of agonists (hormones, neurotransmitters, secretagogues, etc.) to these leads to increase in cAMP or cytosolic calcium concentration. These two second messengers in turn then trigger a variety of cellular processes.

Ethanol could alter the transmembrane signalling processes either by altering the receptor characteristics or receptor binding, or by directly influencing phosphatidyl inositol turnover. Alterations in the neuronal phospholipid metabolism during the development of physical dependence on ethanol were suggested earlier. Littleton and John[34] showed alterations in the fatty acid composition of synaptosomal phospholipids of mouse brain on continuous exposure to ethanol. They found a decrease in the polyunsaturated fatty acid and an increase in the saturated fatty acid content of the membrane phospholipids of mice exposed to ethanol. Since phosphoinositides are rich in arachidonic acid, a significant decrease in its concentration could affect many processes dependent on this fatty acid. It would be interesting to know whether the functions of diacylglycerol formed from phosphoinositides with saturated fatty acid at C-2 are altered. Taraschi et al.[63] observed that multilamellar vesicles composed of recombined phospholipids from ethanol-treated rats were resistant to ethanol-induced fluidization, whereas those from untreated rats were susceptible to such fluidization. When phosphatidylinositol in the vesicles from untreated rats was replaced by this lipid from ethanol-treated rats, the membranes were rendered resistant to such fluidization. All other phospholipids tested were ineffective. This could be due to alterations in the fatty acid moieties of phosphatidylinositol on ethanol treatment. Decreased arachidonic acid and increased oleic acid content in phosphatidylinositol from ethanol-fed rats was in fact observed. Arachidonic acid has been shown to cause a rise in intracellular Ca^{++} in pancreatic islet cells[66]. This was a direct effect of arachidonic acid, and whether such a pathway of Ca^{++} mobilization exists in neuronal cells remains to be seen. Phospholipase A_2 and phospholipid base-exchange enzyme activities of the synaptosomes was shown to be inhibited by the presence of ethanol in incubations in vitro[30]. On the other hand, these enzyme activities were found to be elevated in the synaptosomes from chronically ethanol-treated rats and the addition of ethanol in vitro to these synaptosomal preparations did not alter the enzyme activities, indicating development of tolerance[30]. They concluded that increased phospholipase A_2 activity in the ethanol-treated rat synaptosomes could reduce the proportion of unsaturated fatty acids from membrane phospholipids, as reported earlier[34], and altered phosphatidylserine content, due to enhanced base exchange activity[27], may alter exocytotic release of neurotransmitters.

Ethanol and inositol phospholipid metabolism

In recent years a minor lipid component of the plasma membrane, phosphatidylinositol 4,5-bisphosphate (PIP_2) has emerged as the parent compound, phosphodiesteric hydrolysis of which results in the formation of two intracellular second messengers; namely, myo-inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DG)[12,42]. The former causes a rise in cytosolic Ca^{++} by its mobilization from intracellular nonmitochondrial stores, and the latter stimulates Ca^{++} and phospholipid-dependent phosphorylation of proteins by protein kinase C[4,44]. IP_3 is then quickly hydrolyzed in two steps to inositol 1,4-bisphosphate, inositol 1- or inositol 4-monophosphate, and then to free myo-inositol, which is utilized for the resynthesis of phosphoinositides. Alternatively, IP_3 is also metabolized to inositol 1,3,4,5-tetrakisphosphate and then to inositol 1,3,4-trisphosphate; the function of which is not definitely known yet. DG is either hydrolyzed by lipases or is phosphorylated to phosphatidic acid and is then converted to phosphatidylinositol.

The first indication that the inositol phospholipid metabolism may be altered by ethanol came from the work of Allison and Cicero[1]. They showed significant reduction in myo-inositol 1-phosphate (I-1-P) levels in the cerebral cortex of rats within 5 min following ethanol injection. I-1-P levels returned back to normal values 24 h after the initial injection, and an inverse correlation between blood alcohol and myo-inositol 1-phosphate levels was obtained. However, levels of free myo-inositol in the cerebral cortex are much higher (about 1000-fold), as compared to inositol 1-phosphate, and therefore, alterations in its content may or may not influence the metabolism of phosphoinositides and dependent processes. Alternatively, alcohol may block the synthesis of myo-inositol or its transport across the plasma membrane and can thus affect its metabolism. But Hoffman et al.[26] did not find any significant difference in the amounts of [^{3}H]inositol taken up by brain tissue of chronic ethanol-treated mice, indicating that the transport of myo-inositol is not affected by ethanol. Whether alterations in the inositol phosphate levels occur in chronically alcohol-treated animals is not known. Cortical slices from chronically ethanol-treated rats were found to have only slightly increased phospholipase C activity, as assessed by [^{3}H]inositol phosphate accumulation, but when depolarization-induced [^{3}H]inositol phosphate accumulation was measured, the ethanol-treated group showed significantly increased activity, as compared to controls[28]. The presence of 100 mM ethanol significantly inhibited basal as well as depolarization-induced [^{3}H]inositol phosphate formation in controls, and comparable results were also obtained in the ethanol-treated group[28].

In chronically ethanol-treated rats, $^{32}P_i$ incorporation into phospholipids was studied by Sun et al.[60]. Developing rats were treated with ethanol for 16 days and incor-

poration of $^{32}P_i$ into various synaptosomal phospholipids was analyzed. Phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositols showed an increase in radioactivity in the ethanol-treated group. It was suggested that the increase in the incorporation of $^{32}P_i$ into these phospholipids is an adaptive change in the ethanol-treated animals.

Smith et al.[58] studied the effect of chronic ethanol treatment on receptor-mediated phosphoinositide turnover in mouse forebrain slices. They found that the magnitude of receptor-mediated turnover of phosphoinositides was not altered by ethanol treatment, but muscarinic receptor-stimulated $^{32}P_i$ incorporation into phosphatidic acid, as well as [^{3}H]inositol-1-phosphate accumulation, were inhibited by ethanol added in vitro to the control tissues. Ethanol added in vitro, however, had no effect on the phosphoinositide turnover in tissues from chronically ethanol-treated mice, which suggested the development of tolerance. On the other hand, stimulation of both these processes by norepinephrine and histamine was not influenced by ethanol added in vitro, which indicated that only muscarinic receptors are sensitive to ethanol.

Variable effects of ethanol on agonist-stimulated phosphoinositide metabolism were found in in vitro studies using cerebral cortical slices by Gonzales et al.[19]. They reported a reduction in the basal inositol phosphate level by ethanol at 500 mM, but lower concentrations did not have any effect. Norepinephrine-, potassium-, and glutamate-stimulated [^{3}H]inositol phosphate formation was inhibited by ethanol, but carbachol- and 5-hydroxytryptamine-stimulated [^{3}H]inositol phosphate release was not influenced even at 500 mM ethanol. This is in contrast to the results obtained by Smith et al.[58], who found inhibition of carbachol-stimulated phosphoinositide hydrolysis by ethanol added in vitro to the slices. Variability in the effect of ethanol on receptor-mediated phosphoinositide hydrolysis in various brain regions was also observed by Gonzales et al.[19]. They suggested on the basis of these results that sensitivity of receptor-mediated phosphoinositide metabolism to ethanol is specific for the type of agonist, as well as the brain region. Synthesis of phosphoinositides was not found to be affected by acute or chronic treatment or by in vitro incubation of slices with ethanol, which indicates that ethanol affects only the turnover of these lipids.

Increased numbers of muscarinic cholinergic receptor sites identified on the basis of [^{3}H]QNB binding were reported in the cerebral cortex and hippocampus, but not in the striatum of chronically ethanol-fed mice. These changes in the muscarinic cholinergic receptors were correlated with the phosphoinositide turnover by Hoffman et al.[26]. They found that the EC_{50} for carbachol-stimulation of phosphoinositide breakdown was decreased in the cortex, but had no effect in the striatum of mice chronically treated with ethanol. This was consistent with the observation that QNB binding sites were increased in the cortex, but not in the striatum of these mice. On the other hand, the EC_{50} of carbachol-stimulation of phosphoinositide breakdown, as well as QNB binding, was found to be increased in the hippocampus of these mice[26] owing to an increase in muscarinic binding sites which were not of the M_1 subtype responsible for phosphoinositide turnover. In contrast to Gonzales et al.[19], Hoffman et al.[26] observed an increase in the EC_{50} for carbachol-stimulation of phosphoinositide breakdown at high (500 mM) concentration in vitro. The EC_{50} for carbachol also increased in striatum and hippocampus with 500 mM ethanol[26], but at low ethanol levels the EC_{50} for carbachol did not change in any of these brain regions. However, the EC_{50} for norepinephrine-stimulation was not changed even at high ethanol concentrations in vitro[19,26]. These results suggest that second messenger transmembrane signalling via phosphoinositide breakdown is differentially affected by ethanol in the brain, and regional differentiation in the brain areas also exists.

Receptor regulation of Ca^{++}/Mg^{++}-ATPase

A model for Ca^{++} homeostasis and ethanol sensitivity

Studies from our laboratory and a variety of others suggest that the Ca^{++}/Mg^{++}-ATPase protein may be under receptor regulation by a wide variety of neurohormones and neurotransmitters.

Muscarinic, adrenergic (α_1 and α_2), and opiate agonists have all been shown to produce inhibition of ATPase activity when added in vitro to synaptosomal membrane preparations[16,52,54]. Similarly, LHRH, oxytocin, insulin, and adrenergic agonists have been shown to inhibit Ca^{++}/Mg^{++}-ATPase in pituitary, myometrial, and liver membranes[9,15,46,55]. These findings suggest that receptor control of Ca^{++} homeostasis may provide a major step in the initial rise and prolongation of elevated cytosolic Ca^{++} levels necessary for various intracellular activities, such as ion channel modulation, protein phosphorylation, and secretory events.

The mechanism for this effect is not known, but previous studies suggest that the level of phosphatidyl inositol 4,5-bisphosphate (PIP_2) may play a direct regulatory role in optimal Ca^{++}/Mg^{++}-ATPase sensitivity. In 1982, Penniston[45] suggested that the level of PIP_2 in membranes had a direct bearing on Ca^{++}/Mg^{++}-ATPase activity. Low concentrations of PIP_2 ($< 5\ \mu M$) caused stimulation of enzyme activity over that seen with optimal calmodulin levels. These findings were later confirmed by Choquette et al.[8] and more recently, in our laboratory by Gandhi and Ross[14]. Repeated exposure to membrane stimulation or activation by agonists of the inositol phospholipid cycle would cause a decrease in PIP_2 levels leading to decreased (presumed) Ca^{++}/Mg^{++}-ATPase activity. This is exactly what is found if you observe the activity of the enzyme following exposure to agonists.

In consequence, many agonists which inhibit enzyme activity stimulate hydrolysis of PIP_2^3. The above-reported

studies on muscarinic and adrenergic receptor sensitivity and PIP_2 hydrolysis following ethanol[16,54] show that, following long-term exposure, Ca^{++}/Mg^{++}-ATPase activity may be changed, and this may provide an adaptation mechanism to support Ca^{++} homeostasis and neuronal activity. Ca^{++}/Mg^{++}-ATPase activity is enhanced following chronic treatment with ethanol[47], and as an adaptation response there may be changes in receptor regulation and PIP_2 hydrolysis. We have provided evidence for this using liver plasma membranes following chronic ethanol exposure. Gandhi and Ross[15] demonstrated that basal Ca^{++}-ATPase activity in liver plasma membranes was increased following chronic ethanol exposure. Phenylephrine, an alpha receptor agonist, produced inhibition of ATPase activity in control membranes. However, following chronic ethanol exposure the agonist sensitivity was reversed in such a way that phenylephrine was stimulatory in chronically ethanol-treated membranes. This enhanced Ca^{++} efflux may be part of the adaptation to ethanol, to continue Ca^{++} cycling across the plasma membrane.

Long-term consequences of chronic ethanol administration for the Ca^{++} channels and Ca^{++} pump

Dual regulation by protein kinase-C

In a general sense, there are three areas of focus in studying intracellular Ca^{++} and transmembrane signalling events. One level is the voltage-dependent Ca^{++} channel protein and various subtypes of the Ca^{++} channel, such as the NMDA sensitive subtype (not covered in this paper) and the dihydropyridine-sensitive channel. A second level is the receptor agonist activated phosphatidylinositol 4,5-bisphosphate hydrolysis mechanism to promote inositol tris-phosphate (IP_3) and diacylglycerol (DAG) production[3]. Mobilization of intracellular Ca^{++} by IP_3 provides a stimulus by which intracellular events requiring Ca^{++} are activated. Subsequent activation of protein kinase-C (PK-C) by Ca^{++} and DAG provide additional messenger activities which are directed to ion channels, ion pumps, and autoregulation of phospholipase-C.

The third level of activity centers around Ca^{++} buffering to maintain optimal intracellular levels of Ca^{++} for variable periods of time. The Ca^{++}/Mg^{++}-ATPase represents a primary mechanism for regulating cytosolic levels of Ca^{++} by transfer between the intracellular and extracellular pools[43]. In addition, data presented above provide additional support for the potential role of the Ca^{++}/Mg^{++}-ATPase as a model for the study of ethanol sensitivity and its possible receptor regulation.

Dual control for both ion channels and Ca^{++}/Mg^{++}-ATPase may be under the influence of protein kinase-C. Ca^{++} conductance and [^{3}H]dihydropyridine receptor binding to the Ca^{++} channel protein are both reduced following phorbol ester treatment or application of protein kinase-C directly to preparations[65]. In addition, protein kinase-C may influence K^+ conductance to provide for a dual possibility for ion channel modulation[2].

Recent studies indicate that Ca^{++}/Mg^{++}-ATPase may be directly phosphorylated and activated by protein kinase-C[57]. These investigators used both membrane and soluble Ca^{++}/Mg^{++}-ATPase to demonstrate direct phosphorylation of the Ca^{++} pump and activation of ATP hydrolysis.

Conclusion

Current studies with ethanol demonstrate that acute ethanol treatment decreases Ca^{++}-influx, [^{3}H]dihydropyridine receptor binding, and transmitter release[11,21,37,40]. In addition, acute ethanol treatment also inhibits Ca^{++}/Mg^{++}-ATPase in brain and liver preparations[15,18]. Following chronic ethanol exposure, Ca^{++}-influx, [^{3}H]dihydropyridine receptor number, and Ca^{++}/Mg^{++}-ATPase activity are increased[11,17,20,40,47]. These findings suggest that a form of neuronal adaptation may occur to provide more intracellular Ca^{++} per unit time to continue neuronal activity. The increase in the 'L' type Ca^{++} channel subtype 'L' receptor protein and its functional activity with no change in affinity suggests that new receptor protein is either exposed or activated as a result of ethanol exposure, or that new protein is being synthesized. In order to continue Ca^{++} cycling through intracellular pools, Ca^{++}/Mg^{++}-ATPase activity must subsequently increase as well if this primary buffer mechanism undergoes adaptation. Our findings and those of Keane and Leonard[31] support this hypothesis.

While the mechanism for this increased responsiveness in Ca^{++} channel activity and Ca^{++}-dependent ATP hydrolysis is still unexplored, earlier studies by Hoffman et al. may provide a starting-point. Increased receptor sensitivity and inositol phospholipid turnover may contribute to an increase in intracellular protein kinase-C activation. This could provide one avenue for increased enzyme activity as a result of protein kinase-C stimulation of Ca^{++}/Mg^{++}-ATPase[57]. Long-term changes may result from protein kinase-C anchoring to the membrane, an explanation which may underlie changes in synaptic plasticity and tolerance to drugs of abuse.

In summary, adaptive changes in two Ca^{++} binding proteins, the Ca^{++} channel and Ca^{++}/Mg^{++}-ATPase take place as a result of chronic ethanol exposure. These changes may occur on the basis of alterations in receptor-dependent transmembrane signalling events resulting from increased inositol phospholipid turnover.

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Ethanol and the benzodiazepine-GABA receptor-ionophore complex

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Summary. Ethanol has a pharmacological profile similar to that of classes of drugs like benzodiazepines and barbiturates, which enhance GABAergic transmission in the mammalian CNS. Several lines of behavioral, electrophysiological and biochemical studies suggest that ethanol may bring about most of its effects by enhancing GABAergic transmission. Recently, ethanol at relevant pharmacological concentrations has been shown to enhance GABA-induced ^{36}Cl-fluxes in cultured spinal cord neurons, synaptoneurosomes and microsacs. These enhancing effects of ethanol were blocked by GABA antagonists. Ro15-4513, an azido analogue of classical BZ antagonist Ro15-1788, reversed most of the behavioral effects of ethanol and other effects involving ^{36}Cl-flux studies. The studies summarized below indicate that most of the pharmacological effects of ethanol can be related to its effects on GABAergic transmission.
Key words. Ethanol; GABA receptor complex; chloride channels; Ro15-4513.

The molecular mechanism by which ethanol produces its effects, and the neuronal components involved in ethanol actions, have been a matter of debate over the years. A variety of neuronal pathways have been implicated in various states of alcoholism[48]. However, recent evidence indicates that drugs which have a pharmacological profile similar to ethanol, i.e. benzodiazepines (BZs) and barbiturates, appear to bring about their pharmacological effects by facilitation of inhibitory transmission mediated by GABA. Based on several lines of behavioral, electrophysiological and radioligand-binding studies, and functional assays, it is becoming apparent that ethanol may also mediate many of its effects via GABAergic transmission[17, 53, 58]. This paper will summarize the evidence which implicates $GABA_A$ receptor system in the pharmacological effects of ethanol.

GABA-BZ receptor-ionophore complex

GABA receptor is an oligomeric complex, composed of four binding sites which bear an allosteric relationship to each other (fig. 1). Radioligand binding studies have shown that these sites include GABA recognition sites, BZ sites and picrotoxin sites[38, 51]. Although previous studies suggested that barbiturates act at the picrotoxin sites[56], recent studies based on equilibrium binding and dissociation kinetic studies with [^{35}S]t-butylbicyclophosphorothionate (TBPS), a ligand which binds to the picrotoxin site[39, 45], have shown that barbiturate sites are distinct from the picrotoxin sites[28, 57, 59].

GABA receptor activation opens chloride channels, resulting in hyperpolarizing responses[4, 6]. GABA-mediated responses are blocked by bicuculline, picrotoxin and