Molecular Neurobiology

ISSN0893-7648/98/19(1):1–24/$16.00

Effects of Ethanol on Calcium Homeostasis in the Nervous System

Implications for Astrocytes

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Abstract

Ethanol is a major health concern, with neurotoxicity occurring after both *in utero* exposure and adult alcohol abuse. Despite a large amount of research, the mechanism(s) underlying the neurotoxicity of ethanol remain unknown. One of the cellular aspects that has been investigated in relationship to the neuroteratogenicity and neurotoxicity of ethanol is the maintenance of calcium homeostasis. Studies in neuronal cells and other cells have shown that ethanol can alter intracellular calcium levels and affect voltage and receptor-operated calcium channels, as well as G protein-mediated calcium responses. Despite increasing evidence of the important roles of glial cells in the nervous systems, few studies exist on the potential effects of ethanol on calcium homeostasis in these cells. This brief review discusses a number of reported effects of alcohol on calcium responses that may be relevant to astrocytes' functions.

Index Entries: Ethanol; calcium; astrocytes; nervous system; calcium channels; G proteins; fetal alcohol syndrome; alcohol tolerance.

Introduction

Ethanol is the second most widely abused drug in the world (*1*), with some reports stating that approx 7% of adults in the United States are alcoholics and >20% of hospitalized patients have a medical problem related to drinking (*2*). A recent editorial in *Science* further highlights the societal implications of drinking, citing a 1994 Robert Wood Johnson Foundation report indicating that alcohol abuse costs society nearly $100 billion annually (*3*). Clinically, ethanol exposure can cause neurotoxicity following both acute and chronic intake, and *in utero* exposure can cause permanent damage in the offspring. The acute effects

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of ethanol in humans, as well as the blood alcohol concentrations at which the various effects are seen, have been previously discussed (*4,5*). Briefly, ethanol causes an initial euphoria, presumably through a loss of inhibitory control centers, followed by central nervous system (CNS) depression. As the dose of ethanol increases, impaired cognition, ataxia, memory loss, sedation, coma, and death caused by respiratory arrest can occur. A greater societal problem with respect to alcohol is the development of alcohol dependence or alcoholism. The neurotoxicological effects of chronic alcoholism consist of Wernicke's encephalopathy, which can progress to Korsakoff's syndrome, and include unsteady gait, cerebellar degeneration, and memory impairment (*5*). *In utero* exposure to ethanol can result in the fetal alcohol syndrome (FAS), first described more than 25 yr ago (*6*), whose principal features include growth retardation, specific facial characteristics, and CNS dysfunctions (*7*). The CNS effects are the most devastating feature of FAS, as they are irreversible and persist through adolescence and into adulthood (*8–10*). Among the neuroteratogenic effects of alcohol are microencephaly and mental retardation (*7*), with FAS now considered a leading cause of mental retardation in North America (*11*).

Despite extensive research investigating the CNS effects of ethanol, the mechanisms underlying these effects remain unknown, although many have been proposed. In some instances, ethanol neurotoxicity may be secondary to malnutrition; however, whereas some effects of alcoholism, such as Wernicke syndrome, can be reversed by dietary intervention, others, such as the Korsakoff syndrome, cannot be reversed in this manner (*5*). A great deal of research in animals and in in vitro systems has, however, clearly demonstrated that ethanol can exert direct effects on cells of the nervous system. The cellular effects of ethanol were long thought to be mediated by nonspecific actions at the level of the lipid membrane. In recent years, however, evidence has emerged indicating that the effects of ethanol are more specific than previously thought (*12,13*).

Ethanol has been shown to affect various neurotransmitter receptors, including those of glutamate, acetylcholine, serotonin, and γ-aminobutyric acid (GABA), and to alter specific second messenger systems linked to these receptors (*2,4,14,15*). Certain ion channels, particularly calcium channels, have also been shown to display great sensitivity to the effects of ethanol. Many of these specific effects of ethanol can disrupt the normal cellular calcium homeostasis and calcium responses.

The effects of ethanol on cell calcium have received considerable attention because of the importance of calcium in a variety of cell functions (*16*). Under normal circumstances, intracellular calcium levels are tightly regulated, with cytosolic concentrations maintained around 100 n*M* in the cytosol, with a 10,000-fold calcium gradient across the plasma membrane, and high concentrations of calcium found in the intracellular stores, such as the endoplasmic reticulum and mitochondria (*16*). In response to specific signals, the concentration of calcium in the cytosol can increase many fold to initiate a variety of effects. In neurons, an increase in cell calcium is essential for the release of neurotransmitters, playing a key role in synaptic transmission. Calcium also regulates the function of many enzymes, including some protein phosphatases, protein kinases, and phospholipases (*17*). Calcium has also been shown to regulate its own release from intracellular calcium stores (*18*), and its influx from the extracellular space through calcium channels (*19*). In addition, calcium increases the expression of immediate early genes, such as c-*fos*, c-*myc*, and c-*jun* (*20*), with evidence suggesting that this regulation occurs both posttranslationally (*21,22*) and through the regulation of transcription factors (*22*). There is also evidence that intracellular calcium levels are involved in cellular proliferation (*23,24*) and in both necrotic and apoptotic cell death (*25*).

With the central role that maintenance of calcium homeostasis and calcium responses play in cellular physiology, alterations in calcium may represent an important mechanism for the

neurotoxicity of ethanol. Much of the research on the effects of ethanol on cellular calcium has been done in neuronal, hepatic, or cardiac cells. In recent years, glial cells, once thought of as mere structural supports for neurons, have been established as active players in the development and activity of the CNS. With the discovery of integral roles of glia in the CNS comes the possibility that the function of these cells could be a target for neurotoxicants such as ethanol. The scope of this minireview is to discuss the effects of ethanol on calcium homeostasis, with an emphasis on astrocytes. Because of the limited number of studies carried out in glial cells, relevant data from other systems, especially neuronal preparations, are reviewed and aspects relevant to astrocytes indicated.

Relevance of Ethanol Concentrations

When considering research on the mechanisms underlying the neurotoxicity and neuroteratogenicity of ethanol it is important that the concentrations of ethanol utilized be relevant to the clinical situation. Pharmacokinetic studies have shown that ethanol is distributed rapidly throughout the entire body, with ethanol concentrations in tissues being well approximated by the blood ethanol levels (*26*). Research in pregnant animals has further demonstrated that ethanol concentrations are the same in the maternal blood, fetal blood, and fetal brain after maternal ingestion of ethanol in the guinea pig (*27,28*). It can be assumed that the blood ethanol concentrations (BECs) seen in clinical and animal studies are representative of the levels in the target tissues and are rational concentrations for use in vitro studies.

Legally, many states consider an individual inebriated at a BEC of 100 mg% (21.7 m*M*), and most people will be asleep at 350–400 mg% (76.0–86.8 m*M*) (*29*). Such an evaluation, however, has problems because of the variability of the effects of ethanol between individuals, especially when dealing with chronic alcoholics who have alterations in the metabolism of ethanol caused by enzyme induction and liver damage, and who have developed a tolerance to ethanol. There have been instances where individuals have survived what would normally be considered a exceptionally high or lethal level of ethanol. Survival has been seen in an adolescent with a BEC of 757 mg% (164 m*M*) (*30*) and one case as high as 1.5 g% (325.5 m*M*) (*31*), and ranges of BECs in alcoholics have been reported as 238–489 mg% (51.6–106.1 m*M*) (*32*), and 290–421 mg% (62.93–91.36 m*M*) (*33*). These instances emphasize the high levels of BEC sometimes obtained in the population of concern. Work in animals has also been carried out to determine appropriate levels of ethanol for study, especially for the teratogenic effects of ethanol. Work in guinea pigs demonstrated behavioral impairment and histological effects without gross maternal or fetal toxicity when maternal BECs averaged 54 m*M* (*34,35*). In rats, similar effects were seen at 82.4 m*M* (*36*) and 65.1 m*M* (*37*). Therefore, cellular effects of ethanol at concentrations within the 50- to 100-m*M* range are relevant to concentrations seen in clinical populations. However, because of the high levels that have been seen clinically, cellular effects at concentrations above these levels should not be totally discounted.

Effects of Ethanol on Basal Calcium Levels

As previously mentioned, the cytosolic calcium levels in cells are tightly regulated to maintain a very low basal calcium concentration (*38*). Alterations of basal calcium levels, even if transient, may have detrimental effects on CNS development or functions, or both. A number of studies have investigated the effects of ethanol (alone) on intracellular calcium levels. Different cellular preparations have been used, as well as different exposure protocols. Furthermore, both in vitro and in vivo experiments have been carried out. For the sake of clarity, these studies are discussed based on the duration of ethanol exposure (acute vs short

term vs chronic). The relevance of in vitro findings to in vivo situations is pointed out. Furthermore, potential relevance of these results to glial cells, in particular astrocytes, is discussed.

The data on the ability of acute ethanol exposure to induce an immediate calcium response are inconsistent. In a study using isolated hepatocytes, a response was seen after exposure to moderate levels of ethanol (100–300 m*M*) (*39*), which was dependent on the activation of phospholipase C (PLC). This response in hepatocytes was transient in nature and has been consistently seen in these cells, in studies using both populations of cells as well as individual cells (*40*). Similarly, in PC12 cells, ethanol (400 m*M*) induced a calcium response, although a lower concentration of ethanol (100 m*M*) had no effect (*41*). A lack of effect was also seen in cerebellar granule cells and dissociated brain cells after exposure to a low concentration (50 m*M*) of ethanol (*42,43*). By contrast, ethanol (30–600 m*M*) caused an increase in cytosolic free calcium in rat brain synaptosomes (*44,45*). Furthermore, in contrast to the earlier work in PC12 cells, which showed effects only at high concentrations of ethanol (*41*), more recent work in these same cells demonstrated an ethanol-induced biphasic calcium response, with an increase in calcium levels following exposures of ≤80 m*M* ethanol, and a diminished response at concentrations of > 120 m*M* (*46*). This response to ethanol was inhibited by chelation of extracellular calcium or by blockers of voltage-operated calcium channels, indicating that it is mediated by the influx of extracellular calcium. Webb et al. (*47*) have investigated the effect of ethanol on calcium levels in rat medial septal neurons and septohippocampal neurons using imaging techniques. In medial septal neurons from embryonic 21 rats, low concentrations of ethanol (21.7 m*M*) increased basal calcium in the presence, but not in the absence, of nerve growth factor (NGF) (*47*); however, in postnatal d 0 preparations, this concentration of ethanol had no effect on individual cell basal calcium levels (*48*). In septohippocampal neurons, an increase in intracellular calcium in individual cells was seen following exposure to ethanol under certain conditions, however, as was the case with the medial septal neurons, the responses to ethanol were variable, with no clear concentration–response relationship evident (*49*). Mironov and Hermann (*50*) also demonstrated an effect of ethanol on individual intracellular calcium levels; ethanol, at a concentration as low as 17 m*M*, induced a calcium response in rat hippocampal cells. This response was not dependent on extracellular calcium, but was due to release from nonmitochondrial calcium pools, and involved both inositol 1,4,5-trisphosphate (IP_3) and caffeine-sensitive stores. These researchers also demonstrated that ethanol activates protein kinase C (PKC) and that this activation was necessary for calcium release (*50*).

Short-term (10–30 min) in vitro exposure to ethanol has also been shown to have effects on intracellular calcium levels. Studies by Davidson et al. (*51*) in synaptosomes from rat forebrain, showed that a 10-min preincubation with ethanol (50–500 m*M*) increases calcium levels in a concentration-dependent manner, irrespective of the depolarized state of the cells. In lymphocytes, Fano et al. (*52*) also demonstrated that a 10-min incubation with ethanol (20–200 m*M*) increased basal calcium levels, but they found a bell-shaped concentration-response curve, with a maximum response at 60 m*M* ethanol after a 24-h incubation. By contrast, a short incubation (10–30 min) of skeletal muscle cells with ethanol (20–200 m*M*) resulted in a decrease, rather than an increase, in basal calcium concentration (*53*), and in mixed rat cortical cultures, which were approx 70% glial cells, a 10-min preincubation with ethanol (50–500 m*M*) had no effect on basal calcium concentration, as measured using calcium imaging techniques (*54*). Data from our laboratory using confocal microscopy in both primary rat cortical astrocytes and the human astrocytoma 132 1N1 cell line also indicate that a short incubation (30 min) with ethanol, at concentrations of ≤250 m*M*, has no effect on basal calcium levels (53a).

Chronic exposure (4 d) of PC12 cells to 200 m*M* ethanol caused a PKC-dependent increase

in the uptake of radiolabeled calcium as compared to untreated control cells (*55*). Although the relevance of calcium uptake studies as a marker for increases in intracellular calcium levels has been questioned (*51*), similar results were seen in fetal rat cerebellar preparations, containing predominantly Purkinje neurons, after chronic ethanol treatment (75 m*M*, 48 or 96 h) by directly measuring intracellular calcium levels in single cells (*56*), as well as measurements of currents in PC12 cells after a 6-d exposure to 200 m*M* ethanol (*57*). However, in other imaging studies, ethanol did not affect basal calcium levels in cultured cortical rat neurons after 4 d (100 m*M*) (*58*), or human 132 1N1 astrocytoma cells after 24 h (10–250 m*M*) (*53a*), and decreased basal calcium levels in PC12 cells after a 3-d exposure (*46*). One study carried out in rat astroglial cells (a mixture of astrocytes and oligodendrocytes) found that a 1 week exposure to 50 or 100 m*M* ethanol caused a 50 and 100% increase in intracellular calcium, respectively (*59*).

A few studies have looked at the changes in resting calcium levels after chronic in vivo ethanol treatment. In adult rats, chronic (7 d or 8 wk) in vivo exposure to ethanol did not result in any effect on resting calcium levels (*60,61*). Although few studies have investigated the effects of *in utero* exposure to ethanol on basal calcium levels, one such study, in which blood ethanol concentrations reached 146.3 mg% (30 m*M*) on gestational d 15, showed no effect on basal calcium levels in dissociated whole brain preparations from <1-d-old neonatal rats (*62*).

As can be seen from the results of these studies, which are summarized in Table 1, there is no consistent effect of ethanol on basal cell calcium. Acute ethanol exposure was found to either induce an increase in intracellular calcium levels or to have no effect. The particular response appears to depend on the type of cell studied, and the differentiation and developmental stage of the cells, as well as the concentration of ethanol. In addition, even within the same cell type, contrasting results have been reported, possibly because of differences in culture conditions and experimental techniques. Similarly, short-term and chronic in vitro ethanol treatments have shown great variability in the calcium responses, with increases, decreases, and no effects having been reported. Exposure to ethanol has not been shown to effect calcium levels after either *in utero* or in vivo adult exposure, however, such exposures have not been well studied, and further work needs to be carried out to confirm these results. The variable results which have been reported highlight the importance of carefully studying the effects of ethanol in the cell type of interest, and of being aware of the physiology of the cell type under different culture conditions, and on the mode of exposure to ethanol. Different cell types express different subtypes of voltage-operated calcium channels, as well as different receptors, channels, and pumps in the intracellular stores. Furthermore, even the same cell type will express these components differently depending upon its developmental stage, differentiation state, culturing conditions, with the presence or absence of serum being a major factor. For example, astrocytes express different subtypes of voltage-operated calcium channels under different culturing conditions, and there is a great deal of heterogeneity in these cells even within a given region, let alone from different brain regions and different types of astrocytes (*63*). It should also be noted that after short exposures to high concentrations, or longer exposures to lower concentrations, ethanol could deplete intracellular calcium stores, even though the cytosolic calcium level is constant. This effect could not be detected in the studies discussed, as no studies have looked at specific subcellular locations. This mechanism could be involved in ethanol-inhibited release of calcium from intracellular stores, which remains to be investigated.

Although inconsistent overall, the data indicate that ethanol can affect basal calcium levels in some cell types, either through alterations in calcium influx through voltage-operated calcium channels or through messenger systems

Table 1
Summary of Effects of Ethanol on Basal Calcium Parameters

Exposure type	Effect of ethanol on $[Ca^{2+}]i$[a]	Exposure duration	Concentration of ethanol	Preparation type	References
Acute	↑	<1 min	17–170 m*M*	Rat hippocampal cells	(*50*)
	↑	<1 min	100–300 m*M*	Hepatocytes	(*39,40*)
	↑	<1 min	30–600 m*M*	Rat brain synaptosomes	(*44,45*)
	↑	<1 min	50 m*M*	Cerebellar granule cells	(*42*)
	↔			Dissociated brain cells	(*43*)
	↔ (uptake)	<1 min	45–720 m*M*	Rat brain synaptosomes	(*60*)
	↑	<1 min	≤ 80 m*M*	PC12 cells	(*41,46*)
	↑	<1 min	22–174 m*M*	Untreated rat medial septal neurons	(*47*)
	↓	<1 min	22–174 m*M*	NGF-treated rat medial septal neurons	(*47*)
	↔	<1 min	22–174 m*M*	Untreated rat septohippocampal neurons	(*49*)
	↑	<1 min	22–174 m*M*	NGF-treated rat septohippocampal neurons	(*49*)
Short term	↑	10 min	50–500 m*M*	Rat forebrain synaptosomes	(*51*)
	↑	10 min	20–200 m*M*	Lymphocytes	(*52*)
	↔	10 min	50–500 m*M*	Mixed cortical cultures	(*54*)
	↔	30 min	10–250 m*M*	Cortical astrocytes	(*53a*)
	↔	1–40 min	100 m*M*	PC12 cells	(*41*)
	↓	10–30 min	20–200 m*M*	Skeletal muscle cells	(*53*)
Chronic in vitro	↑ (currents)	4–6 d	200 m*M*	PC12 cells	(*55,57*)
	↑ (uptake)	4–6 d	200 m*M*	PC12 cells	(*55,57*)
	↑	48–96 h	75 m*M*	Fetal rat cerebellar macroneurons	(*56*)
	↑	24 h	20–200 m*M*, max at 60 m*M*	Lymphocytes	(*52*)
	↑	7 d	50–100 m*M*	Rat astroglial cells	(*59*)
	↔	4 d	100 m*M*	Rat cortical neurons	(*58*)
	↔	24 h	10–250 m*M*	Human 132 1N1 astrocytoma cells	(*53a*)
	↔	2 wk	40 m*M*	Skeletal muscle cells	(*53*)
	↓	3 d	100 m*M*	PC12 cells	(*46*)
Chronic in vivo	↔	15 d	29–32 m*M* (BEC)	Neonatal rats	(*62*)
	↔ (uptake)	7 d	7% v/v	Adult rats	(*60*)
	↔ (uptake)	8 wk	32 m*M*(BEC)	Adult rats	(*61*)

[a] Increase levels of intracellular Ca^{2+}, except when indicated.

releasing calcium from intracellular stores. Astrocytes possess voltage-operated calcium channels under certain conditions (*64,65*), as well as IP_3-sensitive calcium stores (*18*). There is also evidence that astrocytes possess ryanodine-sensitive stores, as they respond to stimulation by ryanodine (*66*), and also appear to have caffeine-sensitive responses (*67*). Limited data are available on the affect of ethanol on basal cell calcium in astrocytes, with initial results indicating that at least short incubations with ethanol have no effect on resting calcium levels. Clearly, further studies are needed in these cells to elucidate any action of ethanol on basal calcium levels upon different exposure conditions.

Ethanol and Voltage-Operated Calcium Channels

In addition to maintaining constant calcium levels in the resting state, cells also respond to an array of stimuli with a rise in intracellular calcium levels. A major mechanism by which intracellular calcium concentrations can be increased is by an influx of calcium from the extracellular milieu through voltage-operated calcium channels (VOCCs). Many different subtypes of calcium channels have been identified and the characteristics and distribution of these channels reviewed (*19*). Experimentally, five classes of VOCCs have been distinguished based on biophysical gating characteristics and distinct pharmacology: the T-, L-, N-, P(Q), and R-type VOCCs. Although these channels were once thought to be expressed only in excitable cells, there is now evidence that VOCCs are also found in what were classically considered nonexcitable cells, owing to their lack of ability to respond to electrical stimulation with an action potential (*63*), including astrocytes (*64,68,69*). Because of the role played by VOCCs in calcium responses in both excitable and nonexcitable cells, these channels have been investigated as possible targets of ethanol-induced neurotoxicity.

A large number of studies have been carried out in synaptosomal preparations, brain slices or homogenates, primary neuronal cells, and various cell lines, to investigate the effects of ethanol on VOCCs, using a variety of techniques and tools, including specific channels blockers, binding proteins, and electrophysiology (*70*). This research has focused on alterations of VOCC function after short-term ethanol exposure as a mechanism of the acute neurotoxicity of ethanol and on changes in the density and functions of these channels after chronic ethanol exposure, as a mechanism involved in the pathologies associated with alcoholism and alcohol withdrawal.

Acute ethanol exposure causes an inhibition of calcium influx after activation of VOCCs through depolarization. Earlier studies showed, in both mouse (*60*) and rat (*61*) synaptosomes, that ethanol (25–720 m*M*) inhibited depolarization-induced calcium uptake. Studies by Lynch and Littleton (*71*) showed that in vitro ethanol exposure (50 m*M*) inhibits K^+-stimulated neurotransmitter release by inhibiting calcium influx. In PC12 cells, short-term incubations with ethanol (25 min, at ≥50 m*M* concentrations) decreased radiolabeled calcium uptake following depolarization (*72*); electrophysiological studies in these cells, which contain mainly noninactivating L-type calcium channels, showed an inhibition of calcium currents after 5-min exposure to concentrations as low as 5 m*M* ethanol (*73*). This inhibition did not appear to be attributable to a primary effect on intracellular calcium and its subsequent regulation of channel function, nor to a direct interaction with the high affinity calcium binding site of the channel protein (*73*). Furthermore, undifferentiated PC12 cells were more sensitive to the effects of ethanol than NGF-differentiated cells, which express different levels of the subtypes of calcium channels (*74*). Other electrophysiological studies in dissociated dorsal root ganglions neurons demonstrated that calcium currents are more sensitive to acute inhibition by ethanol than sodium currents, with reversible effects seen after exposure to low doses of ethanol (11–108 m*M*) (*75*).

This sensitivity of calcium channels to low concentrations of ethanol has also been shown in *Aplysia* neurons (*76*), and in two neuroblastoma cell lines, N1E-115 and NG108–15 (*77*). Camacho-Nasi and Treistman (*78*) further demonstrated that these effects in Aplysia neurons are not caused by direct blockage of the channel, but possibly by an action on the channel protein. By contrast, one study looking at the effects of acute in vivo exposure to ethanol reported an increase in the number of binding sites for VOCCs in cerebral cortical, hippocampal and striatal synaptosomes, suggesting that acute ethanol causes an increase in VOCC activity (*79*). This difference could indicate effects specific to in vivo vs in vitro exposure. With the exception of this study, most data indicate that acute ethanol exposure inhibits the influx of calcium through VOCCs, with different selectivity for different subtypes of channels, and that this effect does not appear to be caused by a direct block of the permeation pore itself.

Research on the mechanism(s) by which ethanol may affect channel function points towards GTP-binding proteins (G proteins) as a probable target. Huang and McArdle (*80*) demonstrated that 43.2 m*M* ethanol inhibits calcium currents within 0.5 min and that this effect is inhibited by antibodies that impair the function of the $G_o\alpha$ subunit. Other investigators showed that G_i, a pertussis toxin (PTX)-sensitive G-protein subunit, is involved in the inhibition of L-type, dihydropyridine-sensitive, calcium channels in undifferentiated PC12 cells (*81*). Some VOCCs can also be activated by the addition of MgATP and Ca^{2+}-calmodulin, and inhibition by ethanol of this calmodulin-dependent activation of VOCCs has also been reported (*82*).

In contrast to studies on the actions of acute ethanol exposure, which show an inhibition of channel function, studies on the effects of chronic exposure to ethanol show, for the most part, a tolerance to inhibition by acute ethanol, and an enhancement in channel function or an increase in channel density. Synaptosomes isolated from mice chronically exposed to ethanol were tolerant to inhibition of calcium uptake caused by acute alcohol exposure (*60*). Similar results were seen in synaptosomes from chronically ethanol-treated rats (*61*). In PC12 cells exposed for 6 d to 200 m*M* ethanol, K^+-stimulated calcium uptake was increased (*57,83*), and this increase was inhibited by calcium channel blockers (*83*).

Electrophysiological studies have also shown that in vitro exposure to ethanol alters calcium currents in cerebellar Purkinje neurons from fetal rats. Chronic exposure (8–10 d) to 33 m*M* ethanol enhances high threshold currents; however, low threshold currents are depressed (*84*). In fetal cerebellar macroneurons, however, chronic in vitro ethanol (75 m*M*, 96 h) inhibits the KC1-induced increase in intracellular calcium levels (*56*). These differences could reflect different currents studied or different durations of exposure.

Evidence suggests that the increase in channel function after chronic exposure is attributable to an increase in the number of VOCCs, to compensate for the inhibition seen after acute exposures. In PC12 cells, chronic (2–10 d) ethanol exposure increases both calcium uptake and the number of calcium channels in a reversible manner (*72,85*), possibly through a PKC-dependent mechanism (*55*). Another study also showed that in PC12 cells the increase in calcium uptake is due to increase in L-type channels expression (*86*). In addition, ethanol-dependent rats display an increased number of binding sites for dihydropyridine-sensitive receptors, compared with control rats (*87–89*).

NG 108–15 cells express both L- and N-type calcium channels, and the number of N-type channels increases following differentiation by dibutyryl cyclic adenosine monophosphate (cAMP). In undifferentiated NG 108–15 cells, ethanol (200 m*M*, 72 h) increases the number of L-type channel binding sites but has no effect on the N type; in differentiated cells, however, ethanol does not alter L-type channel numbers, whereas it decreases the number of N-type binding sites in a reversible manner. The effects on the N-type channel are seen only

after 18 h of treatment, are maximal after 72 h, and are reversed 24 h after ethanol withdrawal. With the differences in expression of channels and responses of differentiated and undifferentiated cells, it is important to consider how the cells compare with the in vivo setting, when trying to extrapolate effects (*90*).

Evidence also suggests that the increase in calcium channels plays a role in ethanol withdrawal. In animals, administration of L-type channel blockers, such as nitrendipine and verapamil, prevents the seizures seen after ethanol withdrawal (*91*), and coadministration of calcium channel blockers with ethanol prevents the neuronal adaptations and withdrawal symptoms seen after ethanol exposure and withdrawal in rats (*87*), and mice (*92*). In addition, work in PC12 cells indicates that ethanol may affect the number of calcium channels at a posttranslational level; this effect of ethanol is blocked by PKC inhibitors, implicating this enzyme in the action of ethanol and suggesting a blockage of the normal phosphorylation of the channel as the mechanism by which ethanol is acting (*55*).

In utero exposure to 36% ethanol was shown to affect K^+-stimulated calcium changes, although no selectivity between N-, P-, or L-type channels was seen in whole-brain-dissociated neurons from 1-d-old rats (*62*). This lack of selectivity may indicate a difference in the effect of ethanol between prental and adult exposure, perhaps because of the channels that are present in the adult neurons being more sensitive to the effects of ethanol (*62*).

In summary (Table 2), most studies demonstrate that acute ethanol exposure inhibits the depolarization-induced influx of calcium, whereas chronic ethanol exposure increases this influx by increasing the number of VOCCs; this increase in channel number may also play a role in alcohol withdrawal symptoms. The increase following long-term exposure could be an adaptive response to the inhibition seen following short term exposure. As mentioned, some differences in the effects of ethanol are seen, however, depending on the expression of different subtypes of calcium channels, and the developmental stage of the system studied, and therefore it will be very important to determine the normal channel expression in astrocytes in vivo to interpret results correctly from in vitro studies. Although astrocytes have been shown to express VOCCs under certain conditions (*69*), limited research has been carried out investigating the physiological role of these calcium channels in these cells, or on the ability of ethanol to inhibit their function. Because of the dramatic effects of ethanol on these channels in neuronal cell types, and the role these channels may play in modulating calcium levels in astrocytes, it would be important to investigate the effects of ethanol on these channels in these glial cells.

Ethanol and Receptor-Operated Calcium Channels

In addition to voltage operated calcium channels, other types of calcium channels are expressed in the CNS, including receptor-operated calcium channels (ROCCs). Glutamate, the major excitatory neurotransmitter in the CNS, binds to receptors linked to ROCCs. One subtype of glutamate receptors, the *N*-methyl-D-aspartate (NMDA) receptor, has received much attention as a target of ethanol's neurotoxicity, and there is a great deal of evidence that ethanol can disrupt these receptor-linked channels (*see* reviews *15,93,94*). However, most evidence suggests that astrocytes do not express the NMDA subtype of glutamate receptors (*95*), and therefore, the data on the effects of ethanol on the NMDA receptor are not discussed in this review.

Astrocytes have, however, been shown to express glutamate receptors other than NMDA receptors, including the calcium channel-linked kainate/AMPA receptors (*96*), and these could be a target of ethanol in these cells. A limited amount of work has been done investigating the effects of ethanol on the quisqualate-induced calcium responses, that are mediated by these receptors, and is summarized in Table 3. The effects of acute ethanol on individual cultured Purkinje neurons

Table 2
Summary of Effects of Ethanol on Voltage-Operated Calcium Channels

Type of exposure	Effect of ethanol	Exposure duration	Concentration of ethanol	Preparation type	References
Acute in vitro	↓ Ca^{2+} uptake	30 s–10 min	45–720 m*M*	Mouse synaptosomes	(*60*)
	↓ Ca^{2+} uptake	14 min	25–150 m*M*	Rat synaptosomes	(*61*)
	↓ Ca^{2+}-induced dopamine release	—	50 m*M*	Rat striatal slices	(*71*)
	↓ K^{+}-induced uptake	25 min	50 m*M*	PC12 cells	(*72*)
	↓ L-type Ca^{2+} channel current	5 min	5 or 50 m*M*	PC12 cells	(*73*)
	↓ L-type Ca^{2+} channel current	5 min	25 m*M*	PC12 cells	(*74,81*)
	↓ Ca^{2+} channel current	—	11–108 m*M*	Dorsal root ganglion neurons	(*75*)
	↓ Ca^{2+} channel current	15 min	300 m*M*	*Aplysia* neurons	(*78*)
	↓ Ca^{2+} channel current	15 min	50–500 m*M*	*Aplysia* neurons	(*76*)
	↓ T-type Ca^{2+} channel current	5 min	30–300 m*M*	N1E-115, NG108–15 cells	(*77*)
	↓ L-type Ca^{2+} channel current	5 min	100–300 m*M*	N1E-115, NG108–15 cells	(*77*)
	↑ Ca^{2+} channel current	0.5 min, 10 min	5.4 m*M*	Murine dorsal root ganglion neurons	(*80*)
	↑ Ca^{2+} channel current	0.5 min	43.2 m*M*	Murine dorsal root ganglion neurons	(*80*)
	↓ Ca^{2+} channel current	10 min	43.2 m*M*	Murine dorsal root ganglion neurons	(*80*)
	↓ DHP-sensitive binding sites	10 min	100 m*M*	Rat brain synaptosomes	(*82*)

Chronic in vitro	↑ Ca^{2+} uptake ↑ Ca^{2+} DHP-sensitive binding sites	2–10 d	200 m*M*	PC12 cells	(*55*)
	↑ Ca^{2+} uptake ↑ Ca^{2+} channel current	6 d	200 m*M*	PC12 cells	(*57*)
	↑ Ca^{2+} uptake ↑ DHP-sensitive binding sites	6 d	200 m*M*	PC12 cells	(*72*)
	↑ Ca^{2+} uptake via L-type channels	6 d	200 m*M*	PC12 cells	(*83*)
	↑ Ca^{2+} uptake ↑ DHP-sensitive binding sites	6 d	200 m*M*	PC12 cells	(*85*)
	↑ Ca^{2+} uptake ↑ High threshold currents, ↓ low threshold currents	10–12 min	100–800 m*M*	PC12 cells	(*86*)
	↓ Current	8–10 d	33 m*M*	Fetal rat cerebellar Purkinje cells	(*84*)
	↑ Number of L-type ↔ Number of N-type	96 h	75 m*M*	Fetal rat cerebellar macroneurons	(*56*)
	↔ Number of L-type ↓ Number of N-type	72 h	200 m*M*	Undifferentiated NG108–15 cells	(*90*)
Chronic in vivo	Tolerance	72 h	200 m*M*	Differentiated NG108–15 cells	(*90*)
	Tolerance	7 d	7% v/v	Synaptosomes	(*60*)
	↑ DHP-sensitive binding sites	3 wk	75 m*M* (BEC)	Rat brain synaptosomes	(*82*)
	↑ DHP-sensitive binding sites	8 wk	31.7 m*M* (BEC)	Synaptosomes	(*61*)
	↑ DHP-sensitive binding sites	10 d	27–29 m*M* (BEC)	Cerebral cortex from ethanol-dependent rats	(*87*)
	↑ DHP-sensitive binding sites	6–10 d	Inhalation of 10–22 mg/L	Cortex, heart, vas deferens, and skeletal muscle from ethanol dependent rats	(*88*)
	↑ DHP-sensitive binding sites	4–10 d	Inhalation of 10–15 mg/L	Cortex, heart, and vas deferens from ethanol-dependent rats	(*89*)
In utero	↓ K^+-stimulated Ca^{2+} uptake	15 d	29–32 m*M*	Whole brain-dissociated neurons	(*62*)

appear to be developmentally regulated, with alcohol enhancing the quisqualate-mediated response at early developmental stages, and inhibiting the response in mature neurons (*97*). After chronic (8 d) ethanol treatment, these responses are inhibited in developing cerebellar neurons, as measured by calcium imaging techniques (*98*). These data suggest that ethanol can affect glutamate responses through receptors other than the NMDA receptor and that the response depends on either the duration of ethanol exposure or the specific type of neuron studied. Although no work has been done on astrocytes with respect to these receptors, they should be considered as a possible target for ethanol in these cells. Furthermore, the developmental sensitivity to the effects of ethanol of these responses may be relevant to the teratogenicity associated with ethanol.

In addition to VOCCs and ROCCs, a novel plasma membrane calcium channel was recently described in *Drosophila melanogaster* (*99*) and in some mammalian cell types (*100,101*). These channel proteins, named *trp* or *trp*-like proteins, appear to be involved in the influx of calcium, secondary to second messenger-induced calcium release from intracellular stores (*102,103*). To date, there is no information on either the expression of these channels by astrocytes or the effects of ethanol.

Effects of Ethanol on G-Protein-Mediated Ca^{2+} Changes

In addition to increases in cell calcium caused by calcium influx, activation of G proteins containing the $G\alpha_{q/11}$ subunit by a variety of receptors can lead to elevated cytosolic calcium levels. After G-protein activation, the $G\alpha_{q/11}$ subunit is released and activates PLC, which catalyzes the hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP_2) to IP_3 and diacylglycerol (DAG). DAG activates some isoforms of PKC, whereas IP_3 binds to specific receptors in nonmitochondrial calcium stores and causes the release of calcium into the cytosol (*104*). Any interaction with the phosphoinositide metabolism pathway would result in alterations in calcium homeostasis. A large number of studies exist on the effect of ethanol on the formation of IP_3. In nerve tissue, ethanol does not appear to have any stimulatory effect on phosphoinositide metabolism when present alone (*105*). However, agonist-induced IP_3 formation is mostly inhibited by ethanol (Table 3). For example, muscarinic receptor-stimulated phosphoinositide metabolism was inhibited in cerebral cortical slices (50–500 m*M*) (*105,106*), cortical membranes (250 m*M*) (*107*), mixed rat cortical cultures (approx 70% glia and 30% neurons) (250–500 m*M*) (*54*), and SH-SY5Y neuroblastoma cells (100 m*M*) (*108*). Interestingly, this inhibitory effect appeared to be selective for carbachol-stimulated PI hydrolysis, and to be age dependent, with neonatal rats more sensitive to the action of ethanol than adults (*105,109,110*). Bradykinin-induced PI metabolism was also inhibited by ethanol (100 m*M*) in NG 108–15 neuroblastoma–glioma cells (*111*). Limited studies in astrocytes have shown that acute exposure to ethanol has no effect on norepinephrine (50–200 m*M*) (*112*) or glutamate (100 m*M*) (*113*)-stimulated PI hydrolysis, while it inhibits the muscarinic receptor response (50–250 m*M*) (L. G. Costa, unpublished results). Glutamate-induced PI metabolism was, however, inhibited after a longer exposure to ethanol (100 m*M* for 4 d) (*113*). The varying sensitivity of the neurotransmitter responses to ethanol may be attributable to different second messenger systems downstream from the receptor or to differential sensitivity of the receptor molecule itself.

A few studies have investigated the effects of ethanol on G-protein-mediated intracellular calcium release, all of which indicate that ethanol inhibits the release of calcium from intracellular stores (Table 3). In PC12 cells, a short term incubation with a high concentration (400 m*M*) of ethanol inhibited muscarine-induced increase in intracellular calcium (*41*). Similarly, acute exposure to a low concentration (25 m*M*) of ethanol inhibited carbachol-stimulated calcium uptake in the same cells (*114*). Ethanol also inhibited the carbachol-

Table 3
Summary of Effects of Ethanol on G-Protein-Mediated Receptor Functions

Exposure type	Effect of ethanol	Exposure duration	Concentration of ethanol	Preparation type	References
Acute in vitro	↓ Muscarinic-induced IP_3 response	30 s–90 min	50–500 m*M*	Cerebral cortical slices	(*105,106*)
				Cortical membranes	(*107*)
				Mixed cortical cultures	(*54*)
				SH-SY54 neuroblastoma cells	(*108*)
	↓ Muscarinic-stimulated Ca^{2+} uptake	0–10 min	25–100 m*M*	PC12 cells	(*114*)
	↓ Number of cells with muscarinic-induced Ca^{2+} response	2 min	100 m*M*	SH-SY5Y cells	(*115*)
	↓ size of muscarinic-induced Ca^{2+} response	10 min	250–500 m*M*	Mixed cortical cultures	(*54*)
	↓ Vasopressin-, phenylephrine-, epidermal growth factor-, and angiotensin II-induced Ca^{2+} response	2–3 min	6–300 m*M*	Hepatocytes	(*116,117*)
	↓ Hepatocyte growth factor-induced Ca^{2+} response	2 min	50–300 m*M*	Hepatocytes	(*116,117*)
	↓ Muscarinic-induced Ca^{2+} response	30 min	250 m*M*	132 1N1 astrocytoma cells	(*53a*)
	↑ Metabotropic glutamate receptor-induced response	5 min	33 m*M*	Early developmental stage Purkinje neurons	(*97*)
	↓ Metabotropic glutamate receptor-induced response	5 min	33 m*M*	Mature Purkinje neurons	(*97*)
Chronic in vitro	↓ Bradykinin-induced IP_3 response	4 d	100 m*M*	NG108–15 neuroblastoma–glioma	(*118*)
	↓ Metabotropic glutamate and NE receptor-induced IP_3 response	4 d	100 m*M*	Primary astrocytes	(*113*)
	↓ Metabotropic glutamate receptor-induced response	8–10 d	33 or 50 m*M*	Purkinje neurons	(*98*)
Chronic in vivo	↓ Muscarinic-induced changes	6 d	51–61 m*M* (BEC)	Neonatal rats	(*110*)

induced calcium response in individual SH-SY5Y neuroblastoma cells; ethanol reduced the number of cells that responded to carbachol with an increase in intracellular calcium, although the maximum response and the rate of the calcium response were not affected (*115*). An effect of short-term (10-min) ethanol exposure was also seen on muscarinic receptor-induced calcium responses in mixed cortical cultures using imaging techniques, with a significant inhibition in the size of the maximal response (*54*). Similarly, inhibition of receptor-mediated calcium responses was observed in hepatocytes following stimulation with vasopressin, phenylephrine, epidermal growth factor, angiotensin II (*116*), and hepatocyte growth factor in individual cells (*117*). Confocal microscopy data in human astrocytoma 132 1N1 cells have indicated that a short-term incubation (30 min) with ethanol inhibits the calcium response in these cells, but only at high concentrations (250 m*M*) of ethanol, however, inhibition was seen as low as 25 m*M* after a 24-h incubation with ethanol (*53a*).

These findings indicate that ethanol is capable, in certain situations, to inhibit receptor-activated PI metabolism and the subsequent release of calcium from intracellular stores. The mechanism(s) by which ethanol exerts these effects remain(s) obscure.

Ethanol does not appear to interact directly at the receptor level (*110,112*), suggesting one or more postreceptor targets for its action. Some studies have investigated the effects of ethanol on the activity and levels of the $G\alpha_{q/11}$ G-protein subunit. Results in NG 108–15 cells indicate that chronic ethanol may act at the level of, or distal to, the G protein, possibly at the level of the GDP/GTP exchange on G proteins (*107,118*), and that ethanol decreases $G\alpha_{q/11}$ protein levels (*119*). In addition, a reversible decrease in $G\alpha_{q/11}$ levels was also seen in rat brain after chronic (15-d) in vivo exposure (*120*). In astrocytes, however, functional data indicated an action upstream from G protein (*121*), and chronic ethanol had no effect on the expression of the $G\alpha_{q/11}$ subunit (*122*). These authors suggested that the modulation of $G\alpha_{q/11}$ subunit seen by others (*118,119*) may be specific for neurons.

Downstream from the G-protein, ethanol could act on PLC or at the IP_3 receptor level, to alter the calcium response induced by activation of the PI pathway. Studies in rat brain showed that acute ethanol exposure did not affect PLC levels, but chronic treatment decreased the levels of PLC-β (*120*); by contrast, in primary astrocytes, chronic ethanol increased the levels of PLC-δ1 (*122*). Possible direct effects of ethanol on the IP_3 receptors have also been investigated. Ethanol initially potentiated and then inhibited, both the currents and the calcium changes evoked by caged IP_3 in oocytes (*123*), and, in microsomes, ethanol decreased the amplitude of the calcium released by IP_3, possibly because of selective effects on a particular subtype of IP_3 receptor (*124*). Chronic in vivo exposure to ethanol decreased the number of IP_3 receptors, but not the binding affinity of IP_3 for its receptor in rat cortex (*125*) and mouse Purkinje cells (*126*). These findings suggest that ethanol may also act at the level of the IP_3 receptor to disrupt calcium responses, although this possibility has not been investigated in astrocytes.

Altogether, these data indicate that there are many sites in the inositol phosphate second messenger system where ethanol may act, any one of which would disrupt G-protein-mediated alterations in intracellular calcium. The limited data, however, suggest that the target(s) of ethanol may differ between neurons and astrocytes, highlighting the need to study the effects of this alcohol in different cell types.

Implications for Astrocytes and Alcohol Neurotoxicity

Glial cells comprise much of the volume of the brain, and astrocytes represent an important subtype of glial cell. Although these cell were long thought to be only supporting cells, or "glue", for neurons, several important functions of glial cells are now apparent (*127*). The ability to grow pure (>98%) cultures of astro-

cytes (*128*) has facilitated research on these cells, allowing the complexity of these cells to be realized. It has been demonstrated that astrocytes contain neurotransmitter receptors (*96*), intracellular signaling systems (*129*), and VOCCs (*65*) both in vitro and in vivo.

Glial cells, and specifically astrocytes, are important for the development of the CNS. Astrocytes have been shown to secrete growth factors (*130*), to proliferate in response to some neurotransmitters (*131,132*), to promote dendritic extensions in neurons in vitro (*133*), to be involved in neuronal process elongation (*134*), and to be important in the formation of functioning synapses (*135*). Furthermore, research in *Drosophila melanogaster* has also demonstrated the importance of glial cells during development. Mutation of either the *glial cell missing* (*gcm*) gene or the *reversed polarity* (*repo*) gene resulted in a loss of expression of the glial cell phenotype. These mutated flies did not form appropriate axonal pathways, proper neuronal differentiation did not occur, and there was decreased neuronal survival in late-stage mutant embryos (*136,137*), as well as increased neuronal apoptosis (*138*). A gene homologous to the *gcm* gene, which is a DNA-binding protein, has been found in both the human and mouse, confirming the relevance of this gene in mammalian systems (*139,140*). In the adult CNS, the presence of functional receptors, and the formation of an astrocyte syncytium provide a mechanism by which astrocytes can signal distant cells. The presence of such a signaling system is supported by electrophysiological data in astrocyte cultures (*141*), and by observations of calcium signaling in pure astrocyte cultures (*142*), mixed neuronal–astrocyte cultures (*143,144*), and hippocampal slices (*145*). Astrocyte–neuronal interactions have proved essential for synaptic transmission in hippocampal slices (*146*). Astrocytes have also been shown to receive signals from neurons, as assessed by electrophysiological measures (*147*), and respond to various stimuli with intercellular calcium waves (*142,145,148–150*). This signaling pathway in astrocytes is evidence of the importance of properly functioning astrocytes in the adult CNS.

Although there has been limited research investigating the functional significance of calcium responses in astrocytes, it has been shown that increases in intracellular calcium can have a wide range of effects within cells in general. The role of calcium in the regulation of protein function has long been known, with the activity of phosphatases, kinases, endonucleases, and many other enzymes being calcium dependent. These effects are seen in the role played by calcium in cell death, demonstrating evidence for calcium involvement in both necrosis and apoptosis (*25*). Calcium is also involved in the regulation of protein synthesis through its protein regulatory actions in a variety of cells (*151*). Changes in intracellular calcium levels can have long-term effects on cells through the regulation of immediate-early genes (IEGs), including c-*fos*, c-*myc*, and c-*jun* which act as transcription factors. This calcium regulation of the expression of the IEGs can occur at the level of transcription initiation, elongation of mRNA, the stability of mRNA and at the translational level (*152*). There is evidence that the increased expression of the IEGs seen in many cells after increases in cytosolic calcium can involve both posttranslational effects (*21,22*), and effects at the transcriptional level (*152*). For example, the transcription of c-*fos*, an immediate early gene (IEG) is regulated by the actions of calcium on regulatory proteins that bind to the calcium response element and the serum response element in the upstream region of this gene (*152*). The increase in cell proliferation after cell stimulation by a mitogen is dependent on the induction of IEGs (*23,153*), suggesting that calcium may regulate cellular proliferation through its actions on the IEGs. Calcium has been shown, indeed, to be involved in proliferation in a variety of cell types: it is necessary for proliferation of rat hepatocytes (*154*), the SK-N-MC human neuroblastoma and U-373 MG astrocytoma cell lines (*24*), and rat astrocytes and 132 1N1 human astrocytoma cells (*53a*), and for the entry into the S phase of the

cell cycle in human fibroblasts (*155*), and DDT_1MF-2 smooth muscle cells (*156*).

There is increasing evidence that calcium responses are important in glial cells, as recently reviewed by Verkhratsky et al. (*63*). Astrocytes express VOCCs (*65*) and a wide variety of receptors for neurotransmitters and neuropeptides (*157*), including receptors linked to calcium channels and calcium signaling systems (*132,157–159*). Activation of these receptors in astrocytic cells has been shown to alter calcium levels, and initiate both intracellular and intercellular calcium waves (*142,160,161*). The intracellular calcium response to carbachol in primary astrocytes consists of an initial calcium spike, resulting from the release of calcium from IP_3-sensitive intracellular stores, followed by calcium oscillations and a sustained calcium elevation (*162*). The calcium oscillations and sustained elevation are dependent on the presence of extracellular calcium; similar responses to carbachol are also seen in the human astrocytoma cell line 132 1N1 (*53a*). Although the exact functions of these intracellular calcium responses and intercellular calcium waves are unknown, calcium may act as a signal between neurons and astrocytes, making astrocytes equal partners with neurons in information processing in the brain (*69,163*). The phosphorylation of cAMP response element binding (CREB) protein, and the activation of mitogen-activated protein kinase have been shown to be calcium-dependent in oligodendrocyte progenitor cells (*164*). In addition, it has been recently shown, using an immobilized enzyme preparation, that the frequency of calcium oscillations is crucial in the regulation of calcium- and calmodulin-dependent protein kinase II, demonstrating the sensitivity of cellular enzyme function to calcium levels (*165*). Induction of IEGs has been shown in human astrocytoma cells (*166*) and primary rat astrocytes (*167*) after stimulation with carbachol and in oligodendrocytic cells upon stimulation with glutamate (*168*). The release of interleukin 1 (*169*), and excitatory amino acids (*170,171*) from astrocytes has also been shown to be calcium dependent, as have changes in astrocyte morphology induced by various compounds (*172–174*). The importance of calcium in astrocytes is further underscored by the fact that this ion is thought to act as an intercellular messenger, encoding and transmitting information similar to action potentials in neural networks (*63,69,143,163*).

There is considerable research indicating the widespread importance of calcium in cellular functions. With the increasing evidence of the importance of astrocytes in the development and functioning of the CNS, and the potential role of calcium and calcium signaling in these actions, it is important to consider the control of calcium in astrocytes as a potential target for neurotoxicants, including ethanol. For example, one of the main neuroteratogenic effects of ethanol is microencephaly (*7*); astrocytes comprise a large portion of the volume of the brain and disruption of their proliferation may lead to microencephaly. Ethanol has indeed been shown to inhibit neurotransmitter-induced glial cell proliferation (*175–177*), although the mechanism underlying this inhibition is unknown. Because calcium is thought to participate in cell proliferation (*23,61*) disruption by ethanol may be involved in this inhibitory effect. In addition, ethanol can affect VOCCs, which are present in astrocytes under certain conditions (*65*). The effects of ethanol on these channels in neurons appear to be involved in ethanol's chronic effects in the adult, especially in development of tolerance and withdrawal symptoms (*2*), yet the possible effects of ethanol on these channels have not been studied in astrocytes.

Conclusions

A large number of studies indicate that ethanol can disrupt calcium homeostasis in the nervous system. Significant effects on calcium channels and G-protein-mediated calcium responses have been reported. It is clear, from a review of the available data, that many variables influence the ultimate effect of ethanol, including the developmental stage of the brain, the duration of exposure, the cellular

preparation, and the agonist coupled to a calcium response, to name only a few. Surprisingly, the potential effects of ethanol on calcium homeostasis in astrocytes has not been fully investigated. Yet, it is plausible that, with the important functions of calcium responses in astrocytes, interference by ethanol may mediate some of the neuroteratogenic and neurotoxic effects of this compound.

Acknowledgments

Work by the authors was supported by grants from NIAAA (AA-08154) and NIEHS (ES-07133).

References

1. United States Department of Health and Human Services (1990) *Seventh Special Report to the US Congress on Alcohol and Health.* Secretary of Health and Human Services, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD.
2. Diamond, I. and Gordon, A. S. (1997) Cellular and molecular neuroscience of alcoholism. *Physiol. Rev.* **77,** 1–19.
3. Bloom, F. E. (1997) The science of substance abuse. *Science* **278,** 15.
4. Little, H. J. (1991) Mechanisms the may underlie the behavioural effects of ethanol. *Prog. Neurobiol.* **36,** 171–194.
5. Cotran, R. S., Kumar, V., and Robbins, S. L. (1994) Environmental and nutritional diseases, in *Robbin's Pathologic Basis of Disease,* 5th ed., Saunders, Philadelphia, pp. 379–430.
6. Jones, K. L., Smith, D. W., Ulleland, C. N., and Streissguth, A. (1973) Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet* **7815,** 1267–1271.
7. Clarren, S. K, and Smith, D. W. (1978) The fetal alcohol syndrome. *N. Engl. J. Med.* **298,** 1063–1067.
8. Streissguth, A. P., Aase, J. M., Clarren, S. M., Randels, S. P., LaDue, R. A., and Smith, D. F. (1991) Fetal alcohol syndrome in adolescents and adults. *JAMA* **265,** 1961–1967.
9. Lemoine, P. and Lemoine, P. (1992) Avénir des enfants de mères alcooliques (étude de 105 cas retrouvés à l'age adulte) et quelques constatations d'intérêt prophylactique. *Ann. Pediatr. (Paris)* **39,** 226–235.
10. Spohr, H. L., Willms, J., and Steinhausen, H. C. (1993) Prenatal alcohol exposure and long-term developmental consequences. *Lancet* **341,** 907–910.
11. CCD (Centers of Disease Control) (1995) Trends in fetal alcohol syndrome—United States. *MMWR* **44,** 249–251.
12. Tabakoff, B., Hoffman, P. L., and McLaughlin, A. (1988) Is ethanol a discriminating substance? *Semin. Liver Dis.* **8,** 26–35.
13. Weight, F. F. (1992) Cellular and molecular physiology of alcohol actions in the nervous nystem. *Int. Rev. Neurobiol.* **33,** 289–348.
14. Costa, L. G. (1994) Signal transduction mechanisms in developmental neurotoxicity: The phosphoinositide pathway. *Neurotoxicology* **15,** 19–27.
15. Eggeman, K. T. and Browning, M. D. (1996) Ethanol and the phosphorylation of ligand-gated ion channels, in *Pharmacological Effects of Ethanol on the Nervous System* (Dietrich, R. A., ed.), CRC, Boca Raton, FL, pp. 117–133.
16. Clapham, D. E. (1995) Calcium signaling. *Cell* **80,** 259–268.
17. Ghosh, A. and Greenberg, M. E. (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* **268,** 239–247.
18. Pozzan, T., Rizzuto, R., Volpe, P., and Meldolesi, J. (1994) Molecular and cellular physiology of intracellular stores. *Physiol. Rev.* **74,** 595–636.
19. DeWaard, M., Gurnett, C. A., and Campbell, K. P. (1996) Structural and functional diversity of voltage-activated calcium channels, in *Ion Channels,* (Narahashi, T., ed.), Plenum, New York, pp. 41–60.
20. Werlen, G., Belin, D., Conne, B., Roche, E., Lew, D. P., and Prentki, M. (1993) Intracellular Ca^{2+} and the regulation of early response gene expression in HL-60 myeloid leukemia cells. *J. Biol. Chem.* **268,** 16,596–16601.
21. Sheng, M. and Greenberg, M. E. (1990) The regulation and function of c-*fos* and other immediate early genes in the nervous system. *Neuron* **4,** 477–485.
22. Herschman, H. R. (1991) Primary response genes induced by growth factors and tumor promoters. *Annu. Rev. Biochem.* **60,** 281–319.

23. Kovary, K. and Bravo, R. (1991) The Jun and Fos protein families are both required for cell cycle progression in fibroblasts. *Mol. Cell Biol.* **11,** 4466–4472.
24. Lee, Y. S., Sayeed, M. M., and Wurster, R. D. (1993) Inhibition of human brain tumor cell growth by a receptor-operated Ca^{2+} channel blocker. *Cancer Lett.* **72,** 77–81.
25. Nicotera, P., Zhivotovsky, B., and Orrenius, S. (1994) Nuclear calcium transport and the role of calcium in apoptosis. *Cell Calcium* **16,** 279–288.
26. Lee, N. M. and Becker, C. E. (1992) The alcohols, in *Basic and Clinical Pharmacology* (Katzung B. G., ed.), Appleton & Lange, East Norwalk, CT, pp. 737–771.
27. Clarke, D. W., Steenaart, N. A. E., and Brien, J. F. (1986) Disposition of ethanol and activity of hepatic and placental alcohol dehydrogenase and aldehyde dehydrogenases in the third-trimester pregnant guinea pig for single and short-term oral ethanol administration. *Alcohol. Clin. Exp. Res.* **10,** 330–336.
28. Clarke, D. W., Steenaart, N. A. E., Slack, C. J., and Brien, J. F. (1986) Pharmacokinetics of ethanol and its metabolite, acetaldehyde, and fetolethality in the third-trimester pregnant guina pig for oral administration of acute, multiple-dose ethanol. *Can. J. Physiol. Pharmacol.* **64,** 1060–1067.
29. Snyder, R. and Andrews, L. S. (1996) Toxic effects of solvents and vapours, in *Casarett & Doull's Toxicology. The Basic Science of Poisons* (Katzung B. G., ed.), 5th ed., McGraw-Hill, New York, pp. 737–771.
30. Morgan, D. L., Durso, M. H., Rich, B. K., and Kurt, T. L. (1995) Severe ethanol intoxication in an adolescent. *Am. J. Emerg. Med.* **13,** 416–418.
31. Johnson, R. A., Noll, E. C., and Rodney, W. M. (1982) Survival after a serum ethanol concentration of $1\,^1/_2\%$. *Lancet* **2,** 1394.
32. Jones, A. W. and Sternebring, B. (1992) Kinetics of ethanol and methanol in alcoholics during detoxification. *Alcohol Alcohol.* **27,** 641–647.
33. Adachi, J., Mizoi, Y., Fukunaga, T., Ogawa, Y., and Imamichi, H. (1989) Comparative study on ethanol elimination and blood acetaldehyde between alcoholics and control subjects. *Alcohol. Clin. Exp. Res.* **13,** 601–604.
34. Catlin, M. C., Abdollah, S., and Brien, J. F. (1993) Dose-dependent effects of prenatal ethanol exposure in the guinea pig. *Alcohol* **10,** 109–115.
35. Abdollah, S., Catlin, M. C., and Brien, J. F. (1993) Ethanol neuro-behavioural teratogenesis in the guinea pig: Behavioural dysfunction and hippocampal morphologic changes. *Can. J. Physiol. Pharmacol.* **71,** 776–782.
36. West, J. R., Hamre, K. M., and Cassell, M. D. (1986) Effects of ethanol exposure during the third trimester equivalent on neuron number in rat hippocampus and dentate gyrus. *Alcohol. Clin. Exp. Res.* **10,** 190–197.
37. Moloney, B. and Leonard, B. E. (1984) Pre-natal and post-natal effects of alcohol in the rat. I. changes in body weight and exploratory activity. *Alcohol Alcohol* **19,** 131–136.
38. Finkbeiner, S. M. (1993) Glial calcium. *Glia* **9,** 83–104.
39. Higashi, K., Tsukada, K., Hoshino, M., Nomura, T., Takeuchi, T., and Hoek, J. B. (1994) Inhibition of ethanol-induced inositol phosphate formation and Ca^{2+} mobilization by okadaic acid in rat hepatocytes: Evidence for a role of protein phosphatases in the modulation of phospholipase C by ethanol. *Alcohol Alcohol.* **29,** 53–59.
40. Hoek, J. B., Thomas, A. P., Rooney, T. A., Higashi, K., and Rubin, E. (1992) Ethanol and signal transduction in the liver. *FASEB J.* **6,** 2386–2396.
41. Rabe, C. S. and Weight, F. F. (1988) Effects of ethanol on neurotransmitter release and intracellular free calcium in PC12 cells. *J. Pharmacol. Exp. Ther.* **244,** 417–422.
42. Dildy, J. E. and Leslie, S. W. (1989) Ethanol inhibits NMDA-induced increases in free intracellular Ca^{2+} in dissociated brain cells. *Brain Res.* **499,** 383–387.
43. 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.
44. Daniell, L. C., Brass, E. P., and Harris, R. A. (1987) Effect of ethanol on intracellular ionized calcium concentrations in synaptosomes and hepatocytes. *Mol. Pharmacol.* **32,** 831–837.
45. Rezazadeh, S. M., Woodward, J. J., and Leslie, S. W. (1989) Fura-2 measurement of cytosolic free calcium in rat brain cortical synaptosomes and the influence of ethanol. *Alcohol* **6,** 341–345.
46. Belia, S., Mannucci, R., Lisciarelli, M., Cacchio, M., and Fano, G. (1995) Double effect of ethanol on intracellular Ca^{2+} levels in undifferentiated PC12 cells. *Cell. Signal.* **7,** 389–395.

47. Webb, B., Suarez, S. S., Heaton, M. B., and Walker, D. W. (1995) Ethanol and nerve growth factor effects on calcium homeostasis in cultured embryonic rat medial septal neurons before and during depolarization. *Brain Res.* **701,** 61–74.
48. Webb, B., Suarez, S. S., Heaton, M. B., and Walker, D. W. (1996) Cultured postnatal rat medical septal neurons respond to acute ethanol treatment and nerve growth factor by changing intracellular calcium levels. *Alcohol. Clin. Exp. Res.* **20,** 1385–1394.
49. Webb, B., Suarez, S. S., Heaton, M. B., and Walker, D. W. (1996) Calcium homeostasis in cultured embryonic rat septohippocampal neurons is altered by ethanol and nerve growth factor before and during depolarization. *Brain Res.* **729,** 176–189.
50. Mironov, S. L. and Hermann, A. (1996) Ethanol actions on the mechanisms of Ca^{2+} mobilization in rat hippocampal cells are mediated by protein kinase C. *Brain Res.* **714,** 27–37.
51. Davidson, M., Wilce, P., and Shanley, B. (1988) Ethanol increases synaptosomal free calcium concentration. *Neurosci. Lett.* **89,** 165–169.
52. Fano, G., Belia, S., Mariggio, M. A., Antonica, A., Agea, E., and Spinozzi, F. (1993) Alteration of membrane transductive mechanisms induced by ethanol in human lymphocyte cultures. *Cell. Signal.* **5,** 139–143.
53. Cofan, M., Fernandez-Sola, J., Nicolas, J. M., Poch, E., and Urbano-Marquez, A. (1995) Ethanol decreases basal cytosolic-free calcium concentration in cultured skeletal muscle cells. *Alcohol Alcohol.* **30,** 617–621.
54. 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.
53a. Catlin, M. C., Guizzetti, M., and Costa, L. G. (1999) Effect of ethanol on muscarinic-induced calcium responses in astroglia. In preparation.
55. Messing, R. O., Sneade, A. B., and Savidge, B. (1990) Protein kinase C participates in up-regulation of dihydropyridine-sensitive calcium channels by ethanol. *J. Neurochem.* **55,** 1383–1389.
56. Zou, J. Y., Cohan, C., Rabin, R. A., and Pentney, R. J. (1995) Continuous exposure of cultured rat cerebellar macroneurons to ethanol-depressed NMDA and KCl-stimulated elevations of intracellular calcium. *Alcohol. Clin. Exp. Res.* **19,** 840–845.
57. Grant, A. J., Koski, G., and Treistman, S. N. (1993) Effect of chronic ethanol on calcium currents and calcium uptake in undifferentiated PC12 cells. *Brain Res.* **600,** 280–284.
58. Blevins, T., Mirshahi, T., and Woodward, J. J. (1995) Increased agonist and antagonist sensitivity of *N*-methyl-D-aspartate stimulated calcium flux in cultured neurons following chronic ethanol exposure. *Neurosci. Lett.* **200,** 214–218.
59. Holownia, A., Ledig, M., and Menez, J. F. (1997) Ethanol-induced cell death in cultured rat astroglia. *Neurotoxiol. Teratol.* **19,** 141–146.
60. Harris, R. A. and Hood, W. F. (1980) Inhibition of synaptosomal calcium uptake by ethanol. *J. Pharmacol. Exp. Ther.* **213,** 562–568.
61. Leslie, S. W., Barr, E., Chandler, J., and Farrar, R. P. (1983) Inhibition of fast- and slow-phase depolarization-dependent synaptosomal calcium uptake by ethanol. *J. Pharmacol. Exp. Ther.* **225,** 571–575.
62. Lee, Y. H., Spuhler-Phillips, K., Randall, P. K., and Leslie, S. W. (1996) Effects of prenatal ethanol exposure on voltage-dependent calcium entry into neonatal whole brain-dissociated neurons. *Alcohol. Clin. Exp. Res.* **20,** 921–928.
63. Verkhratsky, A., Orkand, R. K., and Kettenmann, H. (1998) Glial calcium: Homeostasis and signaling function. *Physiol. Rev.* **78,** 99–141.
64. MacVicar, B. A. (1984) Voltage-dependent calcium channels in glial cells. *Science* **226,** 1345–1347.
65. Sontheimer, H. (1994) Voltage-dependent ion channels in glial cells. *Glia* **11,** 156–172.
66. Langley, D. and Pearce, B. (1994) Ryanodine-induced intracellular calcium mobilization in cultured astrocytes. *Glia* **12,** 128–134.
67. Golovina, V. A., Bambrick, L. L., Yarowsky, P. J., Krueger, B. K., and Blaustein, M. P. (1996) Modulation of two functionally distinct Ca^{2+} stores in astrocytes: Role of the plasmalemmal Na/Ca exchanger. *Glia* **16,** 296–305.
68. Sontheimer, H. (1992) Astrocytes, as well as neurons, express a diversity of ion channels. *Can. J. Physiol. Pharmacol.* **70,** S223–S238.
69. Verkhratsky, A. and Kettenmann, H. (1996) Calcium signalling in glial cells. *Trends Neurosci.* **19,** 346–352.
70. Leslie, S. W., Brown, L. M., Dildy, J. E., and Sims, J. S. (1990) Ethanol and neuronal calcium channels. *Alcohol* **7,** 233–236.

71. Lynch, M. A. and Littleton, J. M. (1983) Possible association of alcohol tolerance with increased synaptic Ca^{2+} sensitivity. *Nature* **303,** 175,176.
72. Messing, R. O., Carpenter, C. L., Diamond, I., and Greenberg, D. A. (1986) Ethanol regulates calcium channels in clonal neural cells. *Proc. Natl. Acad. Sci. USA* **83,** 6213–6215.
73. Mullikin-Kilpatrick, D. and Treistman, S. N. (1994) Ethanol inhibition of L-type Ca^{2+} channels in PC12 cells: Role of permanent ions. *Eur. J. Pharmacol.* **270,** 17–25.
74. Mullikin-Kilpatrick, D. and Treistman, S. N. (1995) Inhibition of dihydropyridine-sensitive Ca^{++} channels by ethanol in undifferentiated and nerve growth factor-treated PC12 cells: Interaction with the inactivated state. *J. Pharmacol. Exp. Ther.* **272,** 489–497.
75. Oakes, S. G. and Pozos, R. S. (1982) Electrophysiologic effects of acute ethanol exposure. II. Alterations in the calcium component of action potentials from sensory neurons in dissociated culture. *Dev. Brain Res.* **5,** 251–255.
76. Camacho-Nasi, P. and Treistman, S. N. (1986) Ethanol effects on voltage-dependent membrane conductances: Comparative sensitivity of channel populations in *Aplysia* neurons. *Cell. Mol. Neurobiol.* **6,** 263–279.
77. Twombly, D. A., Herman, M. D., Kye, C. H., and Narahashi, T. (1990) Ethanol effects on two types of voltage-activated calcium channels. *J. Pharmacol. Exp. Ther.* **254,** 1029–1037.
78. Camacho-Nasi, P. and Treistman, S. N. (1987) Ethanol-induced reduction of neuronal calcium currents in *Aplysia:* An examination of possible mechanisms. *Cell. Mol. Neurobiol.* **7,** 191–207.
79. Ruis, R. A., Bergamaschi, S., DiFonso, F., Govoni, S., Trabucchi, M., and Rossi, F. (1987) Acute ethanol effect on calcium antagonist binding in rat brain. *Brain Res.* **402,** 359–361.
80. Huang, G.-J. and McArdle, J. J. (1994) Role of the GTP-binding protein G_o in the suppressant effect of ethanol on voltage-activated calcium channels of murine sensory neurons. *Alcohol. Clin. Exp. Res.* **18,** 608–615.
81. Mullikin-Kilpatrick, D., Mehta, N. D., Hildebrandt, J. D., and Treistman, S. N. (1995) G_i is involved in ethanol inhibition of L-type calcium channels in undifferentiated but not differentiated PC-12 cells. *Mol. Pharmacol.* **47,** 997–1005.
82. Canda, A., Yu, B. H., and Sze, P. Y. (1995) Biochemical characterization of ethanol actions on dihydropyridine-sensitive calcium channels in brain synaptosomes. *Biochem. Pharmacol.* **50,** 1711–1718.
83. Greenberg, D. A., Carpenter, C. L., and Messing, R. O. (1987) Ethanol-induced component of 45 Ca^{2+} uptake in PC12 cells is sensitive to Ca^{2+} channel modulating drugs. *Brain Res.* **410,** 143–146.
84. Gruol, D. L. and Parsons, K. L. (1994) Chronic exposure to alcohol during development alters the calcium currents of cultured cerebellar Purkinje neurons. *Brain Res.* **634,** 283–290.
85. Marks, S. S., Watson, D. L., Carpenter, C. L., Messing, R. O., and Greenberg, D. A. (1989) Comparative effects of chronic exposure to ethanol and calcium channel antagonists on calcium channel antagonist receptor in cultured neural PC12 cells. *J. Neurochem.* **53,** 168–172.
86. Skattebol, A. and Rabin, R. A. (1987) Effects of ethanol on $^{45}Ca^{2+}$ uptake in synaptosomes and in PC12 cells. *Biochem. Pharmacol.* **36,** 2227–2229.
87. Dolin, S. J. and Little, H. J. (1989) Are changes in neuronal calcium channels involved in ethanol tolerance? *J. Pharmacol. Exp. Ther.* **250,** 985–991.
88. Guppy, L. J. and Littleton, J. M. (1994) Binding characteristics of the calcium channel antagonist [^{3}H]-nitrendipine in tissues from ethanol-dependent rats. *Alcohol Alcohol.* **29,** 283–293.
89. Guppy, L. J., Crabbe, J. C., and Littleton, J. M. (1995) Time course and genetic variation in the regulation of calcium channel antagonist binding sites in rodent tissues during the induction of ethanol physical dependence and withdrawal. *Alcohol Alcohol.* **30,** 607–615.
90. Bergamaschi, S., Battaini, F., Trabucchi, M., Parenti, M., Lopez, C. M., and Govoni, S. (1995) Neuronal differentiation modifies the effect of ethanol exposure on voltage-dependent calcium channels in NG 108–15 cells. *Alcohol* **12,** 497–503.
91. Little, H. J., Dolin, S. J., and Halsey, M. J. (1986) Calcium channel antagonists decrease the ethanol withdrawal syndrome. *Life Sci.* **39,** 2059–2065.
92. Whittington, M. A., Dolin, S. J., Patch, T. L., Siarey, R. J., Butterworth, A. R., and Little, H. J. (1991) Chronic dihydropyrindine treatment can reverse the behavioural consequences of

and prevent adaptations to, chronic ethanol treatment. *Br. J. Pharmacol.* **103,** 1669–1676.
93. Crews, F. T., Morrow, A. L., Criswell, H., and Breese, G. (1996) Effects of ethanol on ion channels. *Int. Rev. Neurobiol.* **39,** 283–367.
94. Hoffman, P. L. and Tabakoff, B. (1996) Alcohol dependence: A commentary on mechanisms. *Alcohol Alcohol.* **31,** 333–340.
95. Blankenfeld, G. V., Enkvist, K., and Kettenmann H. (1995) Gamma-Aminobutyric acid and glutamate receptors, in *Neuroglia* (Kettenmann H. and Ranson B. R., eds.), Oxford University Press, New York, pp. 335–345.
96. Porter, J. T. and McCarthy, K. D. (1997) Astrocytic neurotransmitter receptors in situ and in vivo. *Prog. Neurobiol.* **51,** 439–455.
97. Gruol, D. L. and Curry, J. G. (1995) Calcium signals elicited by quisqualate in cultured Purkinje neurons show developmental changes in sensitivity to acute alcohol. *Brain Res.* **673,** 1–12.
98. Gruol, D. L. and Parsons, K. L. (1996) Chronic alcohol reduces calcium signaling elicited by glutamate receptor stimulation in developing cerebellar neurons. *Brain Res.* **728,** 166–174.
99. Hardie, R. C. and Minke, B. (1992) The *trp* gene is essential for a light-activated Ca^{2+} channel in *Drosophila* photoreceptors. *Neuron* **8,** 643–651.
100. Zhu, X., Jiang, M., Peyton, M., Boulay, G., Hurst, R., Stefani, E., and Birnbaumer, L. (1996) *trp,* A novel mammalian gene family essential for agonist-activated capacitative Ca^{2+} entry. *Cell* **85,** 661–671.
101. Philipp, S., Cavalie, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marquart, A., Murakami, M., and Flockerzi, V. (1996) A mammalian capacitative calcium entry channel homologous to *Drosophila* TRP and TRPL. *EMBO J.* **15,** 6166–6171.
102. Hardie, R. C. and Minke, B. (1995) Phosphoinositide-mediated phototransduction in *drosophila* photoreceptors: The role of Ca^{2+} and *trp. Cell Calcium* **18,** 256–274.
103. Minke, B. and Selinger, Z. (1996) Role of *Drosophila* TRP in inositide-mediated Ca^{2+} entry. *Mol. Neurobiol.* **12,** 163–180.
104. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. (1989) Cell signalling, in *Molecular Biology of the Cell,* 2nd ed. (Alberts B., Bray D., Lewis J., Raff M., Roberts K., and Watson J. D., eds.), Garland Publishing, New York, pp. 681–726.
105. Balduini, W. and Costa, L. G. (1990) Developmental neurotoxicity of ethanol: In vitro inhibition of muscarinic receptor-stimulated phosphoinositide metabolism in brain from neonatal but not adult rats. *Brain Res.* **512,** 248–252.
106. Balduini, W., Candura, S. M., Manzo, L., Cattabeni, F., and Costa, L. G. (1991) Time-, concentration-, and age-dependent inhibition of muscarinic receptor-stimulated phosphoinositide metabolism by ethanol in the developing rat brain. *Neurochem. Res.* **16,** 1235–1240.
107. Candura, S. M., Manzo, L., and Costa, L. G. (1992) Inhibition of muscarinic receptor- and G-protein-dependent phosphoinositide metabolism in cerebrocortical membranes from neonatal rats by ethanol. *Neurotoxicology* **13,** 281–288.
108. 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-SY5Y cells. *Biochem. Pharmacol.* **50,** 647–654.
109. Smith, T. L., Yamamura, H. I., and Lee, L. (1986) Effect of ethanol on receptor-stimulated phosphatidic acid and polyphosphoinositide metabolism in mouse brain. *Life Sci.* **39,** 1675–1684.
110. Balduini, W. and Costa, L. G. (1989) Effects of ethanol on muscarinic receptor-stimulated phosphoinositide metabolism during brain development. *J. Pharmacol. Exp. Ther.* **250,** 541–547.
111. Simonsson, P., Sun, G. Y., Vecsei, L., and Alling, C. (1989) Ethanol effects on bradykinin-stimulated phosphoinositide hydrolysis in NG 108-15 neuroblastoma-glioma cells. *Alcohol* **6,** 475–479.
112. 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.
113. Smith, T. L. and Bitrick, M. S. (1995) Lack of involvement of protein kinase C in ethanol-induced inhibition of metabotropic-glutamate receptor function in primary cultures of astrocytes. *Life Sci.* **56,** PL485–489.
114. Koski, G., Lawrence, K., and Righi, D. (1991) Ethanol-induced inhibition of carbachol-stimulated uptake of calcium in PC12 pheochromocytoma cells. *Neuropharmacology* **30,** 267–274.

115. Larsson, C. Ethanol and muscarinic receptor-stimulated signal transduction in nerve cells. in *Ph.D. thesis,* University of Lund, 1995.
116. Higashi, K. and Hoek, J. B. (1991) Ethanol causes desensitization of receptor-mediated phospholipase C activation in isolated hepatocytes. *J. Biol. Chem.* **266,** 2178–2190.
117. Saso, K., Higashi, K., Nomura, T., Hoshino, M., Ito, M., Moehren, G., and Hoek, J. B. (1996) Inhibitory effect of ethanol on hepatocyte growth factor-induced DNA synthesis and Ca^{2+} mobilization in rat hepatocytes. *Alcohol. Clin. Exp. Res.* **20,** 330A–334A.
118. 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.
119. Williams, R. J. and Kelly, E. (1993) Chronic ethanol reduces immunologically detectable $G_{q\alpha/11\alpha}$ in NG108–15 cells. *J. Neurochem.* **61,** 1163–1166.
120. Pandey, S. C. (1996) Acute and chronic ethanol consumption effects on the immunolabeling of $G_{q/11\alpha}$ subunit protein and phospholipase C isozymes in the rat brain. *J. Neurochem.* **67,** 2355–2361.
121. Smith, T. L. (1994) Selective effects of ethanol exposure on metabotropic glutamate receptor and guanine nucleotide stimulated phospholipase C activity in primary cultures of astroctyes. *Alcohol* **11,** 405–409.
122. 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.
123. Ilyin, V. and Parker, I. (1992) Effects of alcohols on responses evoked by inositol trisphosphate in *Xenopus* oocytes. *J. Physiol.* **448,** 339–354.
124. Mezna, M., Patchick, T., Tovey, S., and Michelangeli, F. (1996) Inhibition of the cerebellar inositol 1,4,5-trisphosphate-sensitive Ca^{2+} channel by ethanol and other aliphatic alcohols. *Biochem. J.* **314,** 175–179.
125. Rodriguez, F. D., Lundqvist, C., Alling, C., and Gustavsson, L. (1996) Ethanol and phosphatidylethanol reduce the binding of [^{3}H]inositol 1,4,5-trisphosphate to rat cerebellar membranes. *Alcohol Alcohol.* **31,** 453–461.
126. Simonyi, A., Zhang, J.-P., Sun, A. Y., and Sun, G. Y. (1996) Chronic ethanol on mRNA levels of IP_3R1, IP_3 3-kinase and mGluR1 in mouse Purkinje neurons. *Neuro Report* **7,** 2115–2118.
127. Travis, J. (1994) Glia: The brain's other cells. *Science* **266,** 970–972.
128. McCarthy, K. D. and DeVellis, J. (1980) Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. *J. Cell Biol.* **85,** 890–902.
129. Bhat, N. R. (1995) Signal transduction mechanisms in glial cells. *Dev. Neurosci.* **17,** 267–284.
130. Rudge, J. S., Alderson, R. F., Pasnikowski, E., McClain, J., Ip, N. Y., and Lindsay, R. M. (1992) Expression of ciliary neurotrophic factor and the neurotrophins—nerve growth factor, brain-derived neurotrophic factor and neurotrophin 3—in cultured rat hippocampal astrocytes. *Eur. J. Neurosci.* **4,** 459–471.
131. Ashkenazi, A., Ramachandran, J., and Capon, D. J. (1989) Acetylcholine analogue stimulates DNA synthesis in brain-derived cells via specific muscarinic receptor subtypes. *Nature* **340,** 146–150.
132. Guizzetti, M., Costa, P., Peters, J., and Costa, L. G. (1996) Acetylcholine as a mitogen: muscarinic receptor-mediated proliferation of rat astrocytes and human astrocytoma cells. *Eur. J. Pharmacol.* **297,** 265–273.
133. Tropea, M., Johnson, M. I., and Higgins, D. (1988) Glial cells promote dendritic development in rat sympathetic neurons in vitro. *Glia* **1,** 380–392.
134. Selinfreund, R. H., Barger, S. W., Welsh, M. J., and Van Eldik, L. J. (1990) Antisense inhibition of glial S100β production results in alterations in cell morphology, cytoskeletal organization, and cell proliferation. *J. Cell Biol.* **111,** 2021–2028.
135. Nakanishi, K., Okouchi, Y., Ueki, T., Asai, K., Isobe, I., Eksioglu, Y. Z., Kato, T., Hasegawa, Y., and Kuroda, Y. (1994) Astrocytic contribution to functioning synapse formation estimated by spontaneous neuronal intracellular Ca^{2+} oscillations. *Brain Res.* **659,** 169–178.
136. Hosoya, T., Takizawa, K., Nitta, K., and Hotta, Y. (1995) *Glial cells missing:* A binary switch between neuronal and glial determination in *Drosophila. Cell* **82,** 1025–1036.
137. Jones, B. W., Fetter, R. D., Tear, G., and Goodman, C. S. (1995) *Glial cells missing:* a genetic switch that controls glial versus neuronal fate. *Cell* **82,** 1013–1023.
138. Xiong, W.-C. and Montell, C. (1995) Defective glia induce neuronal apoptosis in the *repo* visual system of *Drosophila. Neuron* **14,** 581–590.

139. Altshuller, Y., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., and Frohman, M. A. (1996) Gcm1, a mammalian homolog of *Drosophila* glial cells missing. *FEBS Lett.* **393,** 201–204.
140. Akiyama, Y., Hosoya, T., Poole, A. M., and Hotta, Y. (1996) The gcm-motif: A novel DNA-binding motif conserved in drosophila and mammals. *Proc. Natl. Acad. Sci. USA* **93,** 14,912–14,916.
141. Murphy, T. H., Blatter, L. A., Wier, W. G., and Baraban, J. M. (1993) Rapid communication between neurons and astrocytes in primary cortical cultures. *J. Neurosci.* **13,** 2672–2679.
142. Cornell-Bell, A. H., Finkbeiner, S. M., Cooper, M. S., and Smith, S. J. (1990) Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science* **247,** 470–473.
143. Charles, A. C. (1994) Glia–neuron intercellular calcium signaling. *Dev. Neurosci.* **16,** 196–206.
144. Nedergaard, M. (1994) Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* **263,** 1768–1771.
145. Dani, J. W., Chernjavsky, A., and Smith, S. J. (1992) Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* **8,** 429–440.
146. Keyser, D. O. and Pellmar, T. C. (1994) Synaptic transmission in the hippocampus: Critical role for glial cells. *Glia* **10,** 237–243.
147. Mennerick, S., Benz, A., and Zorumski, C. F. (1996) Components of glial responses to exogenous and synaptic glutamate in rat hippocampal microcultures. *J. Neurosci.* **16,** 55–64.
148. Charles, A. C., Merrill, J. E., Dirksen, E. R., and Sanderson, M. J. (1991) Intercellular signaling in glial cells: Calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron* **6,** 983–992.
149. Cornell-Bell, A. H. and Finkbeiner, S. M. (1991) Ca^{2+} waves in astrocytes. *Cell Calcium* **12,** 185–204.
150. Enkvist, M. O. K. and McCarthy, K. D. (1992) Activation of protein kinase C blocks astroglial gap junction communication and inhibits the spread of calcium waves. *J. Neurochem.* **59,** 519–526.
151. Palfrey, H. C. and Nairn, A. C. (1995) Calcium-dependent regulation of protein synthesis. *Adv. Second Messenger Phosphoprotein Res.* **30,** 191–223.
152. Rosen, L. B., Ginty, D. D., and Greenberg, M. E. (1995) Calcium regulation of gene expression. *Adv. Second Messenger Phosphoprotein Res.* **30,** 225–253.
153. Kovary, K. and Bravo, R. (1991) Expression of different Jun and Fos proteins during the G0-to-G1 transition in mouse fibroblasts: In vitro and in vivo associations. *Mol. Cell Biol.* **11,** 2451–2459.
154. Bennett, A. M. and Williams, G. M. (1993) calcium as a permissive factor but not an initiation factor in DNA synthesis induction in cultured rat hepatocytes by the peroxisome proliferator ciprofibrate. *Biochem. Pharmacol.* **46,** 2219–2227.
155. Takuwa, N., Zhou, W., Kumada, M., and Takuwa, Y. (1995) Involvement of intact inositol-1,4,5-trisphosphate-sensitive Ca^{2+} stores in cell cycle progression at the G1/S boundary in serum-stimulated human fibroblasts. *FEBS Lett.* **360,** 173–176.
156. Short, A. D., Bian, J., Ghosh, T. K., Waldron, R. T., Rybak, S. L., and Gill, D. L. (1993) Intracellular Ca^{2+} pool content is linked to control of cell growth. *Proc. Natl. Acad. Sci. USA* **90,** 4986–4990.
157. Hosli, E. and Hosli, L. (1993) Receptors for neurotransmitters on astrocytes in the mammalian central nervous system. *Prog. Neurobiol.* **40,** 477–506.
158. Hosli, L., Hosli, E., Maelicke, A., and Schroder, H. (1992) Peptidergic and cholinergic receptors on cultured astrocytes of different regions of the rat CNS. *Prog. Brain Res.* **94,** 317–329.
159. Van Der Zee, E. A., DeJong, G. I., Strosberg, A. D., and Luiten, P. G. M. (1993) Muscarinic acetylcholine receptor-expression in astrocytes in the cortex of young and aged rats. *Glia* **8,** 42–50.
160. Brown-Masters, S., Harden, T. K., and Brown, J. H. (1984) Relationships between phosphoinositide and calcium responses to muscarinic agonists in astrocytoma cells. *Mol. Pharmacol.* **26,** 149–155.
161. Kim, W. T., Rioult, M. G., and Cornell-Bell, A. H. (1994) Glutamate-induced calcium signaling in astrocytes. *Glia* **11,** 173–184.
162. Gafni, J., Munsch, J. A., Lam, T. H., Catlin, M. C., Costa, L. G., Molinski, T. F., and Pessah, I. N. (1997) Xestospongins: Potent membrane permeable blockers of the inositol 1,4,5-trisphosphate receptor. *Neuron* **19,** 723–733.
163. Smith, S. J. (1992) Do astrocytes process neural information? *Prog. Brain Res.* **94,** 119–136.

164. Pende, M., Fisher, T. L., Simpson, P. B., Russell, J. T., Blenis, J., and Gallo, V. (1997) Neurotransmitter- and growth factor-induced cAMP response element binding protein phosphorylation in glial cell progenitors: Role of calcium ions, protein kinase C, and mitogen-activated protein kinase/ribosomal S6 kinase pathway. *J. Neurosci.* **17,** 1291–1301.
165. DeKoninck, P. and Schulman, H. (1998) Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science* **279,** 227–230.
166. Blackshear, P. J., Stumpo, D. J., Huang, J., Nemenoff, R. A., and Spach, D. H. (1987) Protein kinase C-dependent and -independent pathways of protooncogene induction in human astrocytoma cells. *J. Biol. Chem.* **262,** 7774–7781.
167. Arenander, A. T., de Vellis, J., and Herschman, H. R. (1989) Induction of c-*fos* and TIS genes in cultured rat astrocytes by neurotransmitters. *J. Neurosci. Res.* **24,** 107–114.
168. Pende, M., Holtzclaw, L. A., Curtis, J. L., Russell, J. T., and Gallo, V. (1994) Glutamate regulates intracellular calcium and gene expression in oligodendrocyte progenitors through the activation of DL-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *Proc. Natl. Acad. Sci. USA* **91,** 3215–3219.
169. Martin, F. C., Charles, A. C., Sanderson, M. J., and Merrill, J. E. (1992) Subtance P stimulates IL-1 production by astrocytes via intracellular calcium. *Brain Res.* **599,** 13–18.
170. Parpura, V., Basarsky, T. A., Liu, F., Jeftinija, K., Jeftinija, S., and Haydon, P. G. (1994) Glutamate-mediated astrocyte-neuron signalling. *Nature* **369,** 707–708.
171. Jeftinija, S. D., Jeftinija, K. V., Stefanovic, G., and Liu, F. (1996) Neuroligand-evoked calcium-dependent release of excitatory amino acids from cultured astrocytes. *J. Neurochem.* **66,** 676–684.
172. MacVicar, B. A. (1987) Morphological differentiation of cultured astrocytes in blocked by cadmium or cobalt. *Brain Res.* **420,** 175–177.
173. Goldman, R. S., Finkbeiner, S. M., and Smith, S. J. (1991) Endothelin induces a sustained rise in intracellular calcium in hippocampal astrocytes. *Neurocsi. Lett.* **123,** 4–8.
174. Stiene-Martin, A., Mattson, M. P., and Hauser, K. F. (1993) Opiates selectively increase intracellular calcium in developing type-1 astrocytes: Role of calcium in morphine-induced morphologic differentiation. *Dev. Brain Res.* **76,** 189–196.
175. Resnicoff, M., Cui, S., Coppola, D., Hoek, J. B., and Rubin, R. (1996) Ethanol-induced inhibition of cell proliferation is modulated by insulin-like growth factor-I receptor levels. *Alcohol. Clin. Exp. Res.* **20,** 961–966.
176. Guizzetti, M. and Costa, L. G. (1996) Inhibition of muscarinic receptor-stimulated glial cell proliferation by ethanol. *J. Neurochem.* **67,** 2236–2245.
177. Guizzetti, M., Catlin, M., and Costa, L. G. (1997) Effects of ethanol on glial cell proliferation: Relevance to the fetal alcohol syndrome. *Front. Biosci.* **2,** e93–98.