Ethanol Regulates Calcium Channel Subunits by Protein Kinase C δ-dependent and -independent Mechanisms*

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Chronic exposure to ethanol increases the number of functional L-type voltage-gated calcium channels in neural cells. In PC12 cells, this adaptive response is mediated by protein kinase C δ (PKCδ), but the mechanisms by which this occurs are not known. Since expression of several different calcium channel subunits can increase the abundance of functional L-type channels, we sought to identify which subunits are regulated by ethanol. Incubation of PC12 cells with 120–150 mM ethanol for 6 days increased levels of α1C, α2, and β1, subunit immunoreactivity in cell membranes and selectively increased the abundance of mRNA encoding the α1C δ splice variant of α1C. In cells expressing a fragment of PKCδ (δV1) that selectively inhibits PKCδ, there was no increase in membrane-associated α1C, α2, and β1 immunoreactivity following chronic ethanol exposure. However, ethanol still increased levels of α1Cδ mRNA in these cells. These results indicate that ethanol increases the abundance of L-type channels by at least two mechanisms; one involves increases in mRNA encoding a splice variant of α1C and the other is post-transcriptional, rate-limiting, and requires PKCδ.

Voltage-gated calcium channels mediate calcium entry into neurons and regulate neurotransmitter release, firing patterns, gene expression, and differentiation (1, 2). L-type channels are a subfamily of voltage-gated calcium channels that are activated by high voltage, inactivate slowly, and are blocked by dihydropyridines (DHPs). Acute ethanol exposure inhibits the function of L-type channels in several neuronal preparations (3–5). In contrast, in the neural crest-derived cell line PC12, chronic ethanol exposure produces a reversible concentration- and time-dependent increase in K⁺-evoked 45Ca²⁺ uptake and depolarization-evoked calcium currents through L-type channels (6–8). Ethanol-induced increases in L-type channel function are associated with corresponding increases in the density of binding sites for DHP calcium channel antagonists (6, 7), indicating that chronic exposure to ethanol increases the number of functional L-type channels. Similar increases in DHP binding have been detected in brain membranes from ethanol-dependent rodents (9, 10). Up-regulation of L-type calcium channels appears to contribute to intense neuronal hyperexcitability observed during alcohol withdrawal since L channel antagonists reduce tremors, seizures, and mortality in alcohol-dependent rodents deprived of ethanol (11, 12). Ethanol-induced increases in L-type channels may also promote alcohol consumption since L channel antagonists reduce ethanol self-administration in animals (13–16).

Neuronal voltage-gated calcium channels are multimeric complexes of at least three types of subunits as follows: α1, α2δ, and β (2). Diversity within the α1 subunit family is responsible for the major pharmacological and physiological features that distinguish the different classes of calcium channels. α1 subunits contain the calcium pore and binding sites for selective channel antagonists. They are comprised of four homologous repeats (I–IV) each containing six transmembrane segments (S1–S6). Four L-type channel α1 genes have been cloned thus far as follows: α1S from skeletal muscle (17), α1C from heart and brain (18), α1D from neural and endocrine tissues (19), and α1F from retina (20, 21). In brain, α1D and α1C are localized to neuronal cell bodies and proximal dendrites (22). Two splice variants of the rat α1C subunit have been identified, α1C-1 and α1C-2, and are differentially expressed in rat brain (18). The α1C-2 protein differs from the α1C-1 by having a 3-amino acid (aa) insert in the cytoplasmic loop between domains II and III and a 28-aa substitution in the S3 segment in domain IV. In the human α1C gene, this alternatively spliced IV-S3 transmembrane segment is encoded by homologous alternative exons 31 and 32 (23). It is not known if α1C-1, and α1C-2 differ in function.

Protein kinase C (PKC) is a multigene family of phospholipid-dependent, serine-threonine kinases that regulate cell growth and differentiation, neurotransmitter release, receptor regulation, ion channel modulation, and gene expression (24). Twelve PKC isozymes, encoded by 11 genes, have been identified (α, βI, βII, γ, δ, ε, ζ, η, θ, λ, μ, and ν) and differ in structure, requirements for activation, and patterns of expression (24–27). We recently found that ethanol-induced increases in L-type channels can be blocked by an inhibitor of PKCδ (28). In this study we examined the abundance of specific calcium channel subunits and their mRNAs to explore further the mechanisms by which PKCδ mediates up-regulation of L-type channels by ethanol.

EXPERIMENTAL PROCEDURES

Materials—Radioisotopes and nucleotides were purchased from Amersharm Pharmacia Biotech. Restriction endonucleases and modifying enzymes were purchased from Promega (Madison, WI). JM109 (Promega) and XL-1 Blue (Stratagene, La Jolla, CA) bacteria were used. All other reagents were analytical grade and were from Sigma or Life Technologies, Inc.

Cell Culture—PC12 cells (J. Wagner, Cornell University) were cultured at 37 °C in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, 10% horse serum, 50 units/ml penicillin, 50
μg/ml streptomycin, and 2 mM glutamine in a humidified atmosphere of 90% air and 10% CO2. Cells were cultured with 120–150 μM ethanol in tightly capped flasks, and the medium was changed daily as in prior work (28). Control samples were cultured in parallel without ethanol.

**Western Blotting**—For detection of α2 and β subunit immunoreactivity, samples were loaded with PBS, solubilized in 1% digitonin, homogenized in Buffer A containing 10 mM HEPES, pH 7.4, 0.3 mM sucrose, 10 mM EDTA, 10 mM EGTA, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.75 mM benzamide, 0.7 μg/ml peptatin A, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 0.01 μg/ml lysosome, and 8 μg/ml camipain 1→2). Cell membranes were collected by ultracentrifugation at 105,000 × g for 20 min at 4 °C and were resuspended in solubilization buffer containing 300 mM KCl, 150 mM NaCl, 10 mM sodium phosphate, pH 7.4, 10 mM EDTA, 10 mM EGTA, and the protease inhibitors used in Buffer A. Unsolubilized material was removed by centrifugation at 175,000 × g for 45 min at 4 °C. For detection of α1α, and neuronal γ (stargazin [29]) subunit immunoreactivity, cells were treated as described (30). Samples (80, 160, and 320 μg) of crude membrane pellet or digitoxin-solublized membranes were separated on SDS-polyacrylamide gels. Proteins were then transferred to nitrocellulose Hybond C Extra membranes (Amersham Pharmacia Biotech) for 2 h at 100 V, and membranes were incubated in PBS containing 0.1% Tween 20 and 5% nonfat dried milk for 1 h at room temperature. After incubation in primary antibody in the same buffer for 2 h at room temperature, or overnight at 4 °C, the blots were washed to obtain 0.1% Tween 20 for 10 min and then incubated with peroxidase-conjugated goat anti-rabbit IgG (Roche Molecular Biochemicals, 1:1000 dilution) for 1 h in PBS, 0.1% Tween 20 at room temperature. Calcium channel subunit immunoreactivity was detected by chemiluminescence with ECL reagent (Amersham Pharmacia Biotech). Immunoreactive bands were quantified by densitometric scanning, and linear regression analysis was used to determine the slope of the regression line for each condition. Slopes for ethanol-treated samples were divided by slopes calculated for control samples to calculate the percentage increase in expression. Slopes for ethanol-treated samples were divided by slopes calculated for paired control samples to calculate the percentage increase in expression. The first strand of the reaction product, containing α1α, was added to a PCR mixture containing 1.5 mM MgCl2, 100 mM dATP, dGTP, dTTP (dGTP mix) were added. The reaction was then heated for 2 min at 42 °C, and then 1 μl of SuperScript II reverse transcriptase (Life Technologies, Inc.) was added. After incubation for 1 h at 42 °C, 2 μl of the product reaction, containing reverse-transcribed cDNA, was added to a PCR mixture containing 1× polymerase chain reaction (PCR) buffer (Perkin-Elmer), 0.5 μM dNTP mix, 2.5 units of AmpliTaq (Perkin-Elmer), 100 pmol of oligo(dT) primer (Life Technologies, Inc.) and then chilled on ice before 1× first strand buffer (Life Technologies, Inc.), 10 μM dithiothreitol, and 0.5 μM dNTP (dATP, dCTP, dGTP, dTTP) mix were added. The reaction was then heated for 2 min at 37 °C, and then 1 μl of SuperScript II reverse transcriptase (Life Technologies, Inc.) was added. After incubation for 1 h at 42 °C, 2 μl of the product reaction, containing reverse-transcribed cDNA, was added to a PCR mixture containing 1× polymerase chain reaction (PCR) buffer (Perkin-Elmer), 0.5 μM dNTP mix, 2.5 units of AmpliTaq (Perkin-Elmer), 100 pmol of JD1802, and 100 pmol of JD1803. The reaction mixture was then heated to 94 °C for 4 min and then cycled 20 times in an amplification cycle. Each cycle consisted of 53 °C for 45 s, 72 °C for 2 min, and 94 °C for 10 s. After the reaction, the mixture was incubated at 53 °C for 45 s and 2 °C for 2 min and then placed on ice. PCR products were digested with BamHI and HindIII and separated on a 1% agarose gel. The resultant fragments were excised and gel-purified using a QIAEX II Gel Extraction kit (Qiagen, Chatsworth, CA). Purified fragments were subcloned into pBluescript II SK(+) (Strategene, La Jolla, CA), and positive colonies were sequenced. The sequence was identical to the predicted rat α1α, sequence (nucleotides 4046–4628). A HindIII-linearized plasmid was used as a template with T3 RNA polymerase to generate a [α-32P]CTP-labeled 582-bp cRNA probe.

To generate a probe for α1α, two oligonucleotide primers were constructed from the rat α1α, sequence (GenBank accession number M67515) was digested with BglII and PstI, and a 498-bp cDNA fragment (nucleotides 3681–4188) that included the mRNA and α1α, in the IV S3 domain (nucleotides 4098–4181 of α1α, was subcloned into pSP72 (Promega). A BglII-linearized plasmid was used as a template to generate a [α-32P]CTP-labeled 498-bp cRNA probe using SP6 RNA polymerase.

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**RESULTS**

**Chronic Exposure to Ethanol Increases Levels of α1α, α2, and β2 PolylineC7s and L Channel Subunits**—To determine which L-type calcium channel subunits are regulated by ethanol, we first identified which subunits are expressed in PC12 cells. Western analysis of PC12 cell membranes with subunit-specific antibodies demonstrated immunoreactive bands of appropriate molecular mass (32, 33) for α1α, (140 kDa), β1β2 (72 kDa), and β3 (58 kDa) subunits (Fig. 1A). We also found an 86-kDa immunoreactive band using an anti-β2 antibody (Fig. 1A). In a prior report (33), this antibody labeled a 74-kDa protein in Western blots of PC12 cell membranes but recognized 87-, 74-, and 70-kDa protein bands in Western blots of rat cardiac micros.

**Riboprobe Generation**—Full-length rat α1α, cDNA (a gift from T. Snutch, University of British Columbia; GenBank accession number M67515) was digested with BglII and PstI, and a 498-bp cDNA fragment (nucleotides 3681–4188) that included the mRNA and α1α, in the IV S3 domain (nucleotides 4098–4181 of α1α, was subcloned into pSP72 (Promega). A BglII-linearized plasmid was used as a template to generate a [α-32P]CTP-labeled 498-bp cRNA probe using SP6 RNA polymerase.

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Fig. 1. Chronic exposure to ethanol increases levels of $\alpha_{1C}$, $\alpha_2$, and $\beta_{1b}$ polypeptides. A, representative Western blots of membranes isolated from PC12 cells treated for 6 days with or without (control) 150 mM ethanol. Arrow indicates the 72-kDa immunoreactive band. B, membrane-associated $\alpha_{1C}$ immunoreactivity in cells cultured with 150 mM ethanol for 0–6 days. Data are expressed relative to parallel control samples cultured without ethanol and are mean ± S.E. values from four experiments. *, $p < 0.035$ by one-way ANOVA and Fisher LSD tests. C, membrane-associated immunoreactivity for calcium channel subunits in cells cultured for 6 days in 150 mM ethanol. Results are expressed relative to parallel, untreated control samples and are mean ± S.E. values from 3 to 7 experiments. *, $p < 0.05$, two-tailed $t$ test.

Fig. 2. Ethanol-induced increases in $\alpha_{1C}$, $\alpha_2$, and $\beta_{1b}$ subunits are PKCδ-dependent. Antibodies against $\alpha_{1C}$ (A), $\alpha_2$ (B), and $\beta_{1b}$ (C) were used to measure ethanol-induced increases in calcium channel subunits in Western blots of membranes isolated from PC12 cells (PC), vector transfected cells (C), and cells expressing the V1 fragment of PKCδ (V182 and V183). All cultures were treated for 6 days with 150 mM ethanol. Data are expressed relative to parallel control cells cultured without ethanol and are mean ± S.E. values from 3 to 7 experiments. *, $p < 0.036$ by ANOVA and Newman Keuls tests.
FIG. 3. Chronic exposure to ethanol does not alter αδ, β1b, or β1b1 mRNA expression. Abundance of αδ mRNA (A) and β1b mRNA (B) isolated from PC12 cells treated with ethanol for 0–5 days. Data are mean ± S.E. values from three experiments. * p < 0.56 by one-way ANOVA.

ulate basal levels of αC, α2, and β1b, it is required for ethanol-induced increases in these calcium channel subunits.

Ethanol Increases Levels of mRNA for a Splice Variant of αC—Chronic exposure to ethanol alters the abundance of several proteins including tyrosine hydroxylase (42) phospholipase C (43), and the molecular chaperones Hsc 70 (44), GRP 78 (45), and GRP 94 (45) by increasing gene expression. Ethanol-induced increases in L-type channels might also occur at a transcriptional level since PKCα-mediated activation of AP1/Jun-regulated gene expression (46, 47).

To investigate this possibility, we measured the abundance of calcium channel subunits mRNAs in PC12 cells after chronic ethanol exposure. Levels of total RNA per cell were not altered by exposure to 120 mM ethanol for 1–5 days (n = 7, p = 0.3754, ANOVA). Therefore we performed all mRNA studies using total RNA. Subsequent ribonuclease protection assay (RPA) analysis of GAPDH mRNA expression demonstrated that GAPDH mRNAs were not altered by exposure to 120 mM ethanol for 1–5 days (n = 4, p = 0.8742, ANOVA). Therefore, we used GAPDH as an internal control to normalize all subsequent RPAs and slot blots for variation in sample loading. These studies revealed that ethanol did not increase levels of mRNA for αδ, β1, or β1b (Fig. 3).

Two alternatively spliced variants of rat αC have been identified as αC1 and αC2 (18). The major sequence difference between these variants lies within the S3 segment of domain IV between a region that is 43% different at the nucleotide level (36 differences in 84 nucleotides). Therefore, to identify both splice variants simultaneously by RPA, we analyzed αC mRNA using a riboprobe made from the domain IV S3 segment of αC2 that includes this region of mismatch at its 5′ end (Fig. 4A).

This riboprobe was predicted to protect a 498-bp fragment from αC2 mRNA. Mismatch within the 5′ tail of the riboprobe was predicted to yield a fragment that is 84 bp shorter (approximately 414 bp) when complexed with αC1 mRNA. As expected, RPA analysis with this probe revealed two fragments, one approximately 500 bp and the other approximately 410 bp in size. Only the abundance of the shorter fragment was increased by chronic ethanol exposure (Fig. 4B and C). Increases in this putative αC1 transcript were apparent within 1 day of ethanol exposure (p < 0.001, ANOVA, Newman Keuls) and persisted throughout the 6 days of treatment (p < 0.02, ANOVA, Newman Keuls). Upon removal of ethanol from the cultures, the abundance of this mRNA species declined rapidly, reaching base-line levels within 24 h. These findings suggest that chronic exposure to ethanol selectively increases the abundance of αC1 mRNA in PC12 cells.

To ensure that increases in the 410-bp fragment observed with the αC2 probe represent increases in αC1 mRNA, we repeated the RPA analysis with a probe made from αC1 cDNA. As predicted, this probe recognized a major fragment of approximately 582 bp, and exposure to 120 mM ethanol for 0–5 days increased its abundance (data not shown). The magnitude of this increase was similar to that observed for the 410-bp fragment detected with the αC2 probe. This finding confirms our results in Figs. 4 and 5 suggesting that ethanol selectively increases levels of αC1 mRNA.

Ethanol Increases αC1 mRNA Levels in Cells Expressing the δV1 Fragment of PKC—If PKCδ mediates ethanol-induced increases in αC1 mRNA, then αC1 mRNA levels should not be altered in ethanol-treated cells that express δV1. To examine this possibility, we treated V182 cells with 120 mM ethanol for 0–5 days and found that ethanol exposure produced a time-dependent increase in αC1 mRNA abundance (Fig. 5A and B). This increase was also observed in a second δV1-expressing cell line V183 (Fig. 5C). In addition, increases in δV1-expressing cells were much greater than increases observed in PC12 or vector-transfected cells (Fig. 5C). Ethanol did not increase the abundance of αδ, β1b, or β1b1 mRNA in these δV1-expressing cells (Fig. 5D and E). These results indicate that ethanol selectively...
binding, L-type channel function, and \( \alpha_1c, \alpha_2, \) and \( \beta_3 \) subunit immunoreactivities are similar, ethanol-induced increases L-type calcium channels are most likely due to increases in these calcium channel subunits.

Recently we found that increases in DHP binding and L-type channel function following chronic ethanol exposure are inhibited in cells that express the selective PKC\( \delta \) inhibitor, \( \delta V1 \) (28). In the current study, we found that ethanol-induced increases in \( \alpha_1c, \alpha_2, \) and \( \beta_3 \) subunits are also completely blocked in \( \delta V1 \)-expressing cells. Therefore, our present results are consistent with our previous findings and together indicate that PKC\( \delta \) is required for ethanol-induced increases in the density of functional L-type channels in PC12 cells. Our finding that \( \beta_3 \) but not \( \beta_2 \) or \( \beta_\alpha \), was selectively increased by ethanol, suggests that ethanol, via PKC\( \delta \), specifically recruits \( \beta_3 \) subunits to newly formed L-type channel complexes. Exposure to ethanol did not alter \( \beta_3 \) mRNA, indicating that ethanol regulates the abundance of \( \beta_3 \) by post-transcriptional mechanisms. \( \beta \) subunits contain consensus sites for PKC phosphorylation, and phosphorylation of \( \beta \) subunits by PKC has been proposed to regulate L-type channel function (48, 49). Further studies will be required to determine whether PKC\( \delta \) selectively phosphorylates and regulates \( \beta_3 \) protein turnover or trafficking in ethanol-treated cells.

Ethanol exposure also selectively increased mRNA for \( \alpha_1c \) without altering levels of mRNA for the alternative splice variant, \( \alpha_1c_2 \), or for other channel subunits. This is the first report of ethanol regulating the abundance of a specific mRNA splice variant. The most striking difference between \( \alpha_1c_1 \) and \( \alpha_1c_2 \) is a 13-aa substitution within a 28-aa region corresponding to the S3 segment of transmembrane domain IV. Although most of the 13 substitutions within this region are conservative, one exception is the substitution of a proline in \( \alpha_1c_1 \) for an alanine in \( \alpha_1c_2 \) at the amino terminus of the S3 segment. Substitutions located within or near this region may regulate channel gating (50). Electrophysiological studies with expressed \( \alpha_1c_1 \) and \( \alpha_1c_2 \) subunits will be required to investigate this possibility.

Increases in \( \alpha_1c_2 \) were also observed in SV1-expressing cells, suggesting that ethanol increases levels of \( \alpha_1c_2 \) mRNA by PKC\( \delta \)-independent mechanisms. Neither \( \alpha_1c_2 \) nor \( \beta_3 \) mRNA abundance was altered by ethanol treatment in these cell lines, indicating that the response is specific for \( \alpha_1c_1 \). Increases in \( \alpha_1c_1 \) mRNA could be due to ethanol-induced changes in the splicing of \( \alpha_1c \) transcripts leading to greater production of \( \alpha_1c_1 \) mRNA. Alternatively, ethanol may act to decrease \( \alpha_1c_2 \) mRNA degradation. Why these effects of ethanol should be specific for \( \alpha_1c_1 \) mRNA is unknown and requires further study.

In the parent PC12 cell line, \( \alpha_1c_1 \) mRNA levels were increased after 1 day of ethanol exposure and remained nearly constant as long as ethanol was present. In contrast, in \( \delta V1 \)-expressing cells, ethanol induced a much greater rise in \( \alpha_1c_1 \) mRNA, which continued to increase over the 5 days of ethanol exposure. These results suggest that PKC\( \delta \) normally acts to limit \( \alpha_1c_1 \) mRNA abundance, possibly by regulating \( \alpha_1c \) mRNA splicing or by promoting degradation of \( \alpha_1c_1 \) mRNA. This could be due to a direct effect of PKC\( \delta \) on \( \alpha_1c \) mRNA processing or might occur indirectly, if PKC\( \delta \)-mediated increases in calcium channel proteins evoke homeostatic mechanisms that decrease \( \alpha_1c_1 \) mRNA abundance.

Additional splice variants of human \( \alpha_1c \) have been identified (51). Alternative splicing of exons 21 and 22 of the human \( \alpha_1c \) gene produces splice variants in the S2 segment of transmembrane domain III, and these show differences in the voltage sensitivity of inhibition by DHP antagonists (52). Alternative splicing in the cytoplasmic tail alters the kinetics and the calcium dependence of channel inactivation (53). Therefore,

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**FIG. 5.** Ethanol exposure increases mRNA levels of an \( \alpha_1c_1 \) in PC12 and \( \delta V1 \)-expressing cells. A, representative RPA of \( \alpha_1c_1 \) and GAPDH mRNA using the \( \alpha_1c_1 \) cDNA as a labeled probe. Undigested \( \alpha_1c_1 \) and GAPDH mRNA controls (Ua and UG), digested control (D), and V182 mRNA treated for 0–5 days with 120 mM ethanol. B, the abundance of mRNA for \( \alpha_1c_1 \) (C) and \( \alpha_1c_2 \) (○) after exposure to 120 mM ethanol for 0–5 days in V182 cells. Data are expressed as mean ± S.E. values from three experiments. *, \( p < 0.046 \) compared with day 0 by ANOVA and Newman Keuls tests. C, the abundance of \( \alpha_1c_1 \) mRNA after exposure to 120 mM ethanol for 5 days, in PC12 cells (PC), vector transfected cells (C), and cells expressing the V1 fragment of PKC\( \delta \) (V12 and V163). Data are expressed relative to parallel control cells cultured without ethanol and are mean ± S.E. values from three experiments. *, \( p < 0.023 \) compared with PC12 cells or vector-transfected cells (ANOVA and Newman Keuls tests). D, the abundance of \( \alpha_1c_2 \) mRNA after exposure to 120 mM ethanol for 5 days in V162 (Δ) and V163 cells (■) expressing the V1 fragment of PKC\( \delta \). Data are expressed relative to parallel control cells cultured without ethanol and are mean ± S.E. values from three experiments. *, \( p > 0.632 \) by one-way ANOVA. E, the abundance of \( \beta_3 \) mRNA after exposure to 120 mM ethanol for 5 days in V162 (Δ) and V163 (■) cells. Data are expressed relative to parallel control cells cultured without ethanol and are mean ± S.E. values from three experiments. *, \( p > 0.564 \) by one-way ANOVA.

Increases \( \alpha_1c_1 \) mRNA levels by a PKC\( \delta \)-independent mechanism.

**DISCUSSION**

Among the several calcium channel subunits that can contribute to the formation of L-type calcium channels, our clone of PC12 cells appears to express only \( \alpha_1c, \alpha_2, \beta_1b, \beta_2, \) and \( \beta_3 \) subunits. In previous work we found that exposure of PC12 cells to 150 mM ethanol for 6 days increases DHP binding and the function of L-type calcium channels by 55–85% (28). In the current study, we found that exposure to the same concentration of ethanol for the same amount of time increases membrane-associated immunoreactivity for \( \alpha_1c, \alpha_2, \) and \( \beta_3 \) calcium channel subunits by 60–75% without altering immunoreactivity for \( \beta_2 \) or \( \beta_3 \) subunits. Increases in \( \alpha_1c \) immunoreactivity followed a time course similar to that observed previously for increases in K\(^-\)-stimulated \( { }^{45} \text{Ca}^2^+ \) uptake in PC12 cells (7, 28). Since the magnitudes of ethanol-induced increases in DHP
alternative splicing of δ1C, transcripts can confer distinct functional characteristics to neuronal L-type channels. Ongoing studies will investigate whether similar splice variants can be identified in rat neural cells and whether ethanol regulates their abundance.

Our results provide additional evidence for PKCδ as a regulator of L-type channel density and a mediator of cellular adaptation to ethanol. Our findings also indicate that PKCδ acts via post-transcriptional mechanisms to increase the density of L-type channels. In addition, our results provide evidence for a PKCδ-independent mechanism leading to increases in δ1C,1 mRNA that may also contribute to ethanol-induced up-regulation of L-type channels. However, PKCδ-dependent mechanisms appear to be essential and rate-limiting for increases in L-type channels, since inhibition of PKCδ completely prevents ethanol-induced increases in DHP binding and L-type channel function (28). Since antagonists of L-type channels decrease alcohol self-administration (13, 15, 16) and reduce channel function (28), we provide evidence for a PKCδ-dependent mechanism leading to increases in L-type channels. In addition, our results provide evidence for post-transcriptional mechanisms to increase the density of L-type channels. In addition, our results provide evidence for post-transcriptional mechanisms to increase the density of L-type channels.